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EFFECTS OF PUBERTAL STATUS AND NUMBER OF ESTROUS CYCLES PRIOR TO BREEDING IN BEEF HEIFERS, AVERAGE DAILY GAIN ON REPRODUCTIVE PERFORMANCE AND COMPARISON OF TWO FIXED TIME AI ESTRUS SYNCHRONIZATION PROTOCOLS

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by

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EFFECTS OF PUBERTAL STATUS AND NUMBER OF ESTROUS CYCLES PRIOR TO BREEDING IN BEEF HEIFERS, AVERAGE DAILY GAIN ON REPRODUCTIVE PERFORMANCE AND COMPARISON OF TWO FIXED TIME AI ESTRUS SYNCHRONIZATION PROTOCOLS

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University of Nebraska, 2014

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Reproductive success is the most important factor in beef cattle production and is affected by timing of pubertal onset in heifers, and reproductive biotechnologies utilized. Three studies were conducted to evaluate the effects of pubertal status, ADG and two fixed time AI protocols on reproductive success in beef heifers.

In the first study, Heifers that were pubertal prior to breeding had a greater AI and overall pregnancy rate, produced more calves born within the first 21 day of the calving season, and weaned older, heavier calves than non-pubertal heifers. Additionally, number of estrous cycles prior to breeding tended to influence pregnancy rates and heifers that had ≥2 estrous cycles, prior to the first breeding season, had a greater second season pregnancy rate than those heifers that had 0 or 1 estrous cycle prior to the first breeding season.

In the second study, as ADG increased leading up to breeding the odds of attaining puberty increased for GSL heifers. For NP heifers, as ADG increased the odds

of puberty attainment decreased. Odds of pregnancy were affected by body weight gain and pubertal status interaction; however, pubertal status had the greatest influence on increasing the odds of pregnancy.

In the third study, two progestin-based fixed time AI protocols, MGA and 14-d CIDR, were compared to evaluate pregnancy rates. Fixed time AI pregnancy rate and final pregnancy rate was similar between MGA and 14-d CIDR. An economic analysis was performed and determined the synchronizing heifers with MGA was more cost-effective in this study.

In summary, if a heifer attains puberty prior to the breeding season acceptable pregnancy rates can be achieved regardless of the number of estrous cycles experienced prior to breeding. However, second season pregnancy rates may be affected. Additionally, MGA and 14-d CIDR produce similar and acceptable fixed time AI pregnancy rates.

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Chapter I: LITERATURE REVIEW

Introduction

Beef cow-calf operations' productivity and profitability is dependent on the development of beef heifers as replacement breeding animals for the cow herd. Profitability is directly influenced by a cow's reproductive lifespan and her ability to wean a marketable calf each year. In order for replacement heifers to be generated with these characteristics, producers must produce a pregnant heifer early in the first breeding season without excessive development costs and manage the heifer to return to estrus and become pregnant early in the following breeding seasons. This is important as it takes 3 to 5 weaned calves to recover the development costs for a replacement heifer.

Multiple factors contribute to optimal heifer reproductive performance, but first and foremost is achievement of puberty prior to the first breeding season. Development of heifers to have 1 to 3 estrous cycles prior to their first breeding season is the industry standard, as heifers are more likely to become pregnant when they have had 3 estrous cycles prior to breeding compared to having only one estrous cycle (Byerley et al., 1987). The management tools that impact age at puberty are genetics and nutrition. However, selection for accelerated genetics for reproductive traits may take several generations to see results, whereas proper nutrition can produce more immediate results in attainment of puberty and fertility.

Research has shown that heifers of similar breed composition can reach puberty several months apart when developed on different diets (Wiltbank et al., 1969; Short and Bellows, 1971). However, the differences in age at puberty due to differing nutritional regimes comes with great financial impact, as 60 to 70% of heifer development costs are attributed to feed. Therefore, the cost of an earlier age at puberty needs to be weighed against the profits to be made by increased pregnancy rates and heavier weaning weights.

In addition, there are many technologies that can be implemented in a management system to help induce puberty, have more heifers achieve pregnancy early in the breeding season, and inseminate heifers with semen from proven sires for calving ease or low birth weights. Two of these reproductive biotechnologies are estrus synchronization and AI. They have been available for more than 30 years, however producers have been slow to adopt them. Perhaps this is due to labor intensity and costs associated with estrus synchronization pharmaceuticals and semen for AI; however, labor intensity is dramatically decreased with the use of fixed-time AI (FTAI), while still producing acceptable pregnancy rates. Again, the use of these technologies can hasten puberty onset in peri-pubertal heifers, concentrate the breeding period and calving period, reduce incidences of dystocia if high accuracy, low birth weight bulls are utilized.

Estrous Cycle

The cyclical pattern of ovarian activity that facilitates sexual receptivity, allowing for mating, and establishment of pregnancy is called an estrous cycle (Forde et al., 2011). Cattle have an estrous cycle that consists of 2 phases: the luteal phase and the follicular phase. The luteal phase is the period following ovulation through the formation and lifespan of the corpus luteum. The follicular phase is the period from the regression of the corpus luteum to ovulation (Forde et al., 2011). These phases can also be broken down into 4 stages, proestrus and estrus, which occur during the follicular phase, and metestrus and diestrus make up the luteal phase.

The average length of the estrous cycle in cattle is 21 d; however, the length is determined by the number of follicular waves, which varies between individual cows. Cattle most commonly have 2 or 3 follicular waves with a new wave starting every 6 to 8 d, therefore the estrous cycle can range from 18 to 24 d (Figure 1).

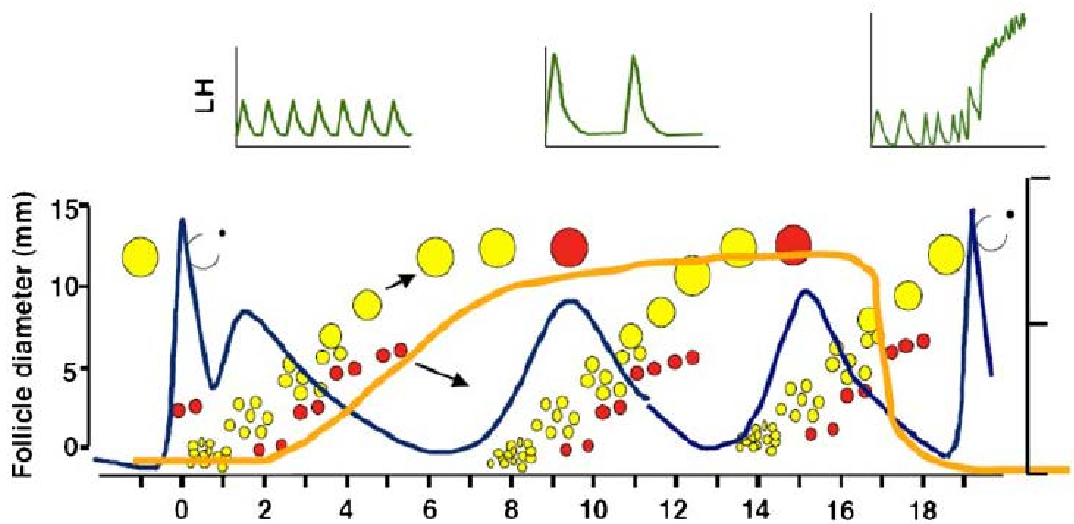

Figure 1. The illustration above depicts the follicular waves that occur throughout the bovine estrous cycle. The yellow follicles are healthy growing follicles, whereas the red follicles are atretic. Additionally, patterns of follicle stimulating hormone (FSH; blue), luteinizing hormone (LH; green) and progesterone (P4; orange) are depicted. Ovulation is induced by a surge of LH and FSH. (Adapted from Forde et al., 2011).

The estrous cycle is under endocrine regulation of the hypothalamic-pituitary-gonadal axis (HPG) and functions under positive and negative feedback systems, which differs from the follicular phase to the luteal phase.

Follicular Phase

Proestrus is the stage of the estrous cycle that leads to estrus, where ovulation occurs. During proestrus, progesterone concentration decreases. Lower progesterone concentration decreases the negative feedback progesterone plays on the hypothalamus, increasing GnRH release; as well as the anterior pituitary, increasing secretion of gonadotropins, LH and FSH. The increase in GnRH allows for stimulation of gonadotropin release, allowing for follicular growth, thus increased concentration of estrogen (Figure 2). According to Roche (1996), when serum progesterone levels are basal and LH pulses occur every 40 to 70 minutes.

Estrus is the stage of the estrous cycle that follows proestrus, in which estrogen concentrations are continuing to rise until a threshold concentration or peak is reached. The peak concentration of estrogen (positive feedback to the hypothalamus) causes a large quantity of GnRH to be released from the surge center, which stimulate the anterior lobe of the pituitary to secrete the preovulatory surge of LH (Sunderland et al., 1994). The preovulatory surge of LH is at least 10 times greater than the tonic pulses of LH. The LH surge causes ovulation of the dominant follicle. Ovulation occurs approximately 10 to 14 h after the observed standing estrus.

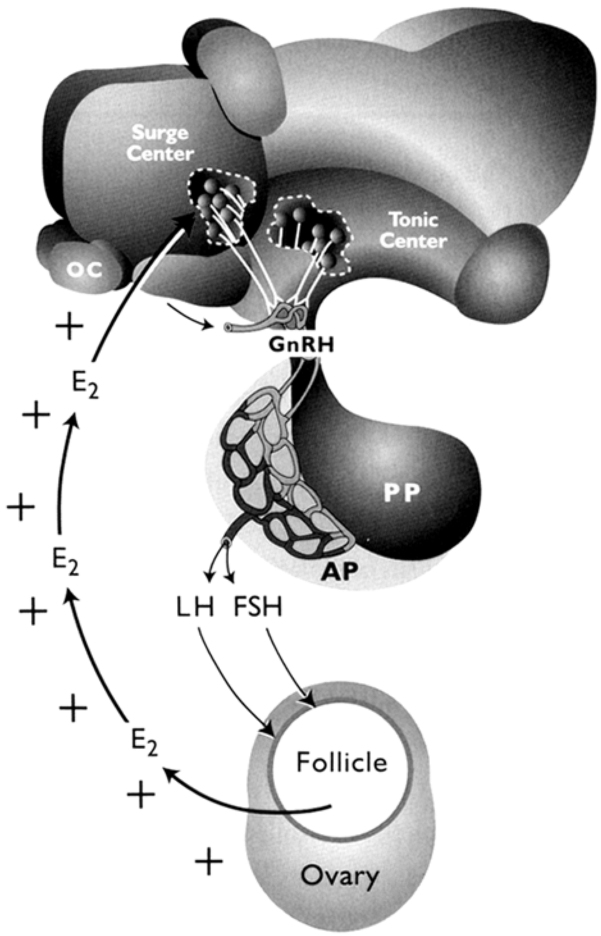

Figure 2. The figure above illustrates the hypothalamic-pituitary-gonadal (HPG) axis during the follicular phase of the estrous cycle. The positive feedback mechanism of estrogen (E_2) on the surge center of the hypothalamus is depicted, stimulating a surge release of gonadotropin releasing hormone (GnRH), thus stimulating the pre-ovulatory surge of lutenizing hormone (LH) from the anterior pituitary. (Adapted from Senger, 2012).

Luteal Phase

Metestrus, the stage of the estrous cycle that follows estrus, is the beginning of the luteal phase. Metestrus occurs during the first 5 d of the estrous cycle (ovulation being d 0) and formation of the corpus luteum (CL) occurs during this stage. Once the follicle ruptures during ovulation, blood vessels within the follicular wall also rupture, giving the area on the ovary where the follicle was located a bloody appearance, known as a corpus hemmorhagicum. When the ruptured follicle collapses, it causes many folds, where the cells of the theca interna and granulosa cells begin to mix, forming a gland that consists of connective tissue, theca cells and granulosa cells (Senger, 2012). Theca cells and granulosa cells then undergo a dramatic transformation in to luteal tissue called lutenization, which is governed by LH. This structure becomes the CL and starts to produce progesterone. Progesterone is the hormone responsible for preparing the uterus for implantation and maintaining pregnancy.

During diestrus, the CL continues to grow for the first few days, then reaches its maximum growth and progesterone secretion peaks and remains constant for approximately 10 d (Figure 3). Progesterone being secreted by the CL exhibits a negative feedback on GnRH secretion from the hypothalamus. During the luteal phase, estrogen also exhibits negative feedback on the hypothalamus and anterior pituitary. The negative feedback that occurs during the luteal phase prevents ovulation from occurring when the HPG is under control of progesterone. If recognition of a pregnancy (interferon-τ) is not detected by d 18 of the estrous cycle, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is produced by the uterus to regress the CL and decrease progesterone concentrations produced (luteolysis), which occurs the last 2 days of diestrus. Luteolysis is the irreversible degradation of the CL, thus causing a decrease in progesterone concentration and removing the negative feedback.

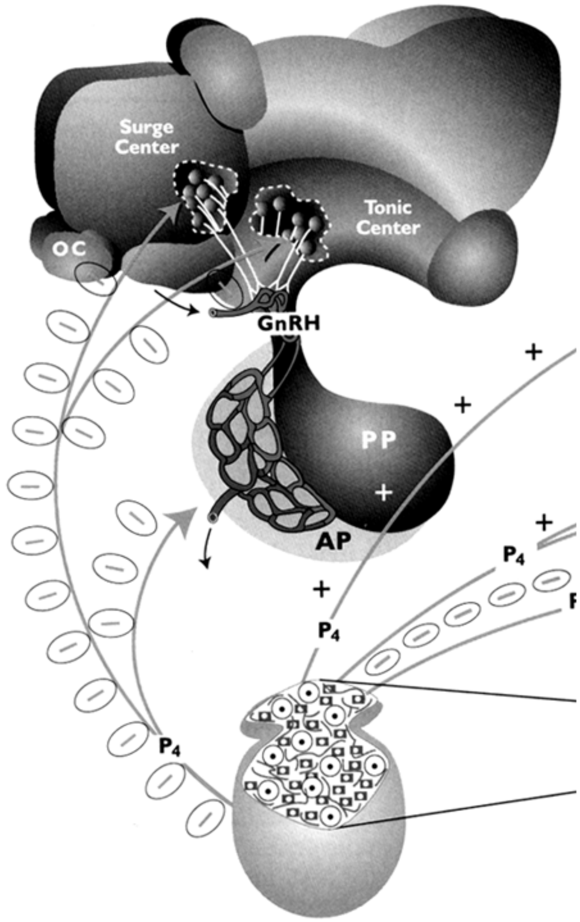

Figure 3. The figure above illustrates the hypothalamic-pituitary-gonadal (HPG) axis during the luteal phase of the estrous cycle. The negative feedback of progesterone (produced by the corpus luteum on the ovary) on hypothalamus and anterior pituitary are depicted. (Adapted from Senger, 2012).

During the luteal phase, recurrent waves of follicle development continue; however, the negative effect of the high level of progesterone does not allow LH to be secreted at a frequency high enough to cause ovulation, therefore those follicles become atretic (Rahe et al., 1980).

Puberty

Normal estrous cycles, with ovulation of a dominant follicle and luteal phase of 15 to 17 d in length, do not occur in heifers until after they have reached puberty. Puberty is defined as the first ovulatory estrus followed by a luteal phase of normal length (15 to 17 d) and is the first opportunity for a heifer to conceive (Atkins et al., 2013). Estrus and ovulation can occur independently of each other in peri-pubertal heifers. Therefore, non-pubertal estrus or ovulation without estrus should not be confused with puberty, where ovulation is accompanied by behavioral estrus.

The part of the endocrine system that regulates the onset of puberty is the HPG. Prior to puberty, GnRH neurons within the hypothalamus are highly sensitive to a strong negative inhibition by estradiol; however, the negative effect of estradiol decreases as heifers mature and approach puberty. Changes in GnRH neuron sensitivity to estradiol initiates puberty, allowing for GnRH to be released at the appropriate frequency and quantity to stimulate gonadotropin secretion. Gonadotropins, LH and FSH, are produced and released by the anterior pituitary. Follicle stimulating hormone causes follicular growth, an increase in estradiol secretions as follicle diameter increases, and an increase in LH pulse frequency, leading to an LH surge and ovulation (Kinder et al., 1995; Day and Anderson, 1998).

Throughout development of the HPG in heifers, the amount of GnRH in the hypothalamus does not change, only the sensitivity of GnRH neurons changes (Kinder et al., 1995). No morphological changes have been observed in GnRH neurons, however changes in estrogen receptor and kisspeptin within the hypothalamus have been identified as major players in puberty initiation (Anderson and Day, 1996).

Within the hypothalamus GnRH neurons do not contain estrogen receptors (ER); however, ER are located in many other areas within the hypothalamus, such as medial preoptic area (MPOA), anterior hypothalamus (AH), ventrolateral septum, bed nucleus of stria terminalis, ventromedial hypothalamus (VMH), and the arcuate nucleus (ARC; Day and Anderson, 1998; Atkins et al., 2013). Neurons that are ER-positive decrease in the AH and medial basal hypothalamus (MBH) as puberty approaches, which has been reported to be negatively correlated with LH pulse frequency (Day et al., 1987).

The exact mechanisms that stimulate the change in sensitivity of GnRH neurons to estradiol have not been elucidated, but metabolic signals seem to be involved. The age at which beef females reach puberty can be affected by many factors, some of particular interest are weight, plane of nutrition, and ADG prior to and following weaning.

Reproductive tract development

Prenatal development

Reproductive organs start to develop well before birth in beef heifers, with ovary development occurring by d 50 to 60 of gestation and primordial follicles identified on fetal bovine ovaries by d 74 to 80 of gestation (Hubbert et al., 1972; Tanaka et al., 2001; Nilsson and Skinner, 2009). Concentrations of FSH and estradiol have been detected around mid-gestation in the bovine fetus, which continue to increase throughout the duration of gestation (Tanaka et al., 2001). The maturation of reproductive tissues and the endocrine axis continues after birth.

Postnatal development

Reproductive development,which was initiated in-utero, continues through the peri-pubertal stage in beef heifers, with wave-like patterns of follicular development being observed as early as 2 wk of age in heifer calves (Gasser, 2013). Honaramooz et al. (2004) evaluated the reproductive tissues of Hereford heifers every 2 wk from 2 to 60 wk after birth via transrectal ultrasonography. From this study, important phases of growth were identified in beef heifers. Ovarian dimensions increased from 2 to 14 wk and again after 34 wk. The size of the largest ovarian follicles increased from 8 to 14 wk, 38 to 42 wk, and 52 to 60 wk. The number of follicles ≥3 mm in diameter tended to increase from 6 to 14 wk and significantly increased from 6 to 60 wk of age. The first ovulation occurred, on average, at 63.7 wk of age. The heifers in this study were gaining BW at an average of approximately 2 lb/d throughout this study with heifer BW at puberty averaging 883 lb.

This study gives insight on how nutrition during some of these time points could influence reproductive tract development, and potentially times of development to put more emphasis on nutrient intake. It is known that reproductive development starts in-utero, so attention should be paid to dam nutrition during gestation. Additionally, from the birth to 14 wk and after 34 wk ovarian development occurs, growth in diameter and number of follicles, meaning these may be times of development to put more research focus on to see how nutrition during these periods affect age at puberty and number of antral follicles.

Pre-weaning management

Many studies have provided evidence that diet during development can account for some of the physiological changes necessary for the attainment of puberty (Frisch, 1984). A hormone called leptin, may be one of the main players in effect that diet has on puberty initiation. Leptin is a hormone that signals nutritional status to the HPG; its expression and secretion have been correlated with body fat mass (Zieba et al., 2005). Heifers of similar breed composition can reach puberty several months apart when fed different diets (Wiltbank et al., 1969; Short and Bellows, 1971). In a study looking at BW and age at puberty in Hereford heifers done by Arije and Wiltbank (1974) found heifers that had a greater growth rate from birth to weaning, heavier weaning weights and reached puberty earlier, at a heavier BW than their herd mates with a slower growth rate prior to weaning. Additionally, they found those heifers that grew more rapidly after weaning tended to be heavier at puberty, but not necessarily younger.

Wiltbank et al. (1966) found that regardless of overwinter feeding treatment, pre-weaning ADG had a significant effect on age at puberty, with a 0.1 kg increase in ADG leading to an 18.7 d decrease in age at puberty. When evaluating the effect of post-weaning ADG on age at puberty, it only had a significant effect if heifers were on a low level of nutrition over winter. Wiltbank et al. (1966) determined that age at puberty was more consistently affected by preweaning BW gain versus post-weaning BW gain.

Gasser (2013) reviewed multiple studies that focused on the peripubertal period in heifers. These studies included early weaning and feeding a high concentrate diet to heifers. These heifers had a substantially reduced age at puberty, increased ovarian maturation, and increased estradiol concentrations during follicular waves. This research provides evidence that nutritional influence during the preweaning can greatly influence timing of puberty, and thus reproductive performance.

Well-controlled studies on pre-weaning nutrition are limited; many studies have estimated pre-weaning growth rate and weaning weight (Hall, 2013). The dams milking ability may be the largest factor contributing to the importance of pre-weaning growth and its role in fertility. Maternal milk production influences calf weaning weight, which research has shown plays a role in puberty (Corah et al., 1975). Additionally, Gasser et al. (2006) have shown nutrient status within the first 2 to 3 months of age impacts the onset of puberty.

Post-weaning management

For approximately 20 years, industry standards have been to develop replacement heifers to 65% of their mature BW in order to ensure attainment of puberty prior to the breeding season (Patterson et al., 1992). Many studies were conducted to determine whether puberty was controlled by age or BW, and studies have shown that rate and timing of BW gain can influence age at puberty (Wiltbank et al., 1966; Arije and Wiltbank, 1974; Lynch et al., 1997). However, research in the past decade has challenged the 65% of mature BW rule. As feed costs have increased, it has been important to evaluate how much nutrition is actually required to maintain heifer reproductive performance.

Currently heifers are developed to reach puberty by 12 to 15 months of age so they can conceive and calve as a 2-yr old. Byerley et al., (1987) discovered the fertility to the first estrus was 21 percentage points less than heifers inseminated on the third estrus. Therefore, it has been an industry standard to develop heifers to attain puberty 1 to 3 months prior to the breeding season. With the increased cost of feedstuffs it is important that emphasis is put on nutrition during critical growth periods to allow heifers to attain puberty in time to have multiple estrous cycles prior to the breeding season.

It is still in question if pre-weaning or post-weaning nutrition has a greater influence on heifer reproductive performance. Roberts et al. (2009) nutrient restricted heifers for 140 d after weaning, by doing this the proportion of heifers that attained puberty by 14 months was decreased; however, by the end of the breeding season pregnancy rates were similar between the restricted heifers and the control heifers. In another study done by Funston and Deutscher (2004), heifers were developed to either 53% or 58% of their mature BW with no significant differences in calving interval, calving date, or pregnancy rate through the third breeding season. (Short and Bellows, 1971) fed heifers to gain either 0.45 kg/d or 0.68 kg/d. Heifers that gained .68 kg/d achieved puberty approximately one month earlier than those that gained 0.45 kg/d.

One postweaning study done by (Funston and Larson, 2011) looked at 2 post-weaning heifer development systems, a traditional drylot development system (DL) and an extensive grazing system (EXT) utilizing crop residue and winter range. The heifers in the DL system were heavier throughout the breeding season (387 vs. 336 kg) and a greater proportion of the DL heifers were pubertal before the breeding season (88 vs. 48%); however, by the end of the breeding season pregnancy rates were similar between DL and EXT heifers.

Earlier studies suggest postweaning gain has a greater influence on heifer age at puberty and pregnancy rates, than what has been shown in more recent research. Genetic advancements that have taken place over the past couple decades, such as selection for increased scrotal circumference that is negatively correlated with age of puberty in female progeny, may be playing a large part in the changes seen over time in attainment of puberty and heifer fertility.

Estrus Synchronization

Estrus synchronization is one of the most important and advantageous reproductive techniques that has been available for use by cattle producers for several decades. The advantages to using estrus synchronization are that estrus occurs during a predicted time range which allows for AI, embryo transfer, or other reproductive techniques to be utilized. This provides the opportunity for more females to conceive earlier in the breeding season, thus leading to a more concentrated calving season and calves being more uniform in age and weight at weaning. Research has shown that calves born within the first 21 d of the calving season are older and heavier at weaning and have greater lifetime productivity than their contemporaries born later in the calving period (Lesmeister et al., 1973; Funston et al., 2012). By utilizing estrus synchronization, with or without AI, females have a greater opportunity to become pregnant early in the breeding season, thus will calve earlier in the calving season.

Estrus synchronization was developed in 6 phases, as researchers began to understand the mechanisms that controlled the estrous cycle in cattle. The first discovery was that progesterone inhibited ovulation (Ulberg et al., 1951) and understanding the maturation of pre-ovulatory follicles (Hansel et al., 1961; Lamond, 1964). Therefore, the initial protocols of estrus synchronization development centered on control of the luteal phase, as the follicular waves had yet to be recognized. The Progesterone Phase, was the first phase, where exogenous progesterone was administered to cattle to prolong an existing or establish an artificial luteal phase. The second phase, Progesterone-Estrogen, used estrogens and gonadotropins to manipulate the estrous cycle. The third phase, PG phase, came about in 1972 when prostaglandin and its analogs were found to be luteolytic in cattle (Lauderdale, 1972). The combination of progesterone, estrogens, and prostaglandin comprises the fourth phase of development.

Through ultrasonography, Sirois and Fortune (1988) discovered the bovine estrous cycle is comprised of distinct wave-like patterns of follicular growth occurring anywhere from 6 to 15 d apart. Once this was discovered the goal was to find a synchronization method that allowed control of both the follicular waves and the luteal phase, therefore the GnRH-PG phase was initiated. The GnRH-PG protocols worked effectively in increasing synchronization rate in beef cattle compared to methods in the previous phases (Twagiramungu et al., 1992a; Twagiramungu et al., 1992b). Administering GnRH initiates a new follicular wave 2 to 3 d following administration, the luteal tissue that forms following GnRH injection is capable of lutetolysis via PG injection 6 to 7 d later (Twagiramungu et al., 1995). A percentage of cattle were observed coming into estrus between injections of GnRH and PG, therefore stimulating interest in the sixth phase of estrus synchronization, Progestogen-GnRH-PG.

Melengestrol Acetate

Melengestrol acetate (MGA) is an orally active synthetic progestin developed in 1962 (Patterson et al., 1989). This progestin has greater hormone activity with 125 times more effective than progesterone (Lauderdale et al., 1983) and MGA binds the bovine progesterone receptor with 5.3 times greater affinity than its physiological ligand. It is used to synchronize estrus in heifers, as it is only FDA approved for use in heifers. Melengestrol acetate must be consumed by heifers at a rate of .5mg/hd/d in order for MGA to suppress estrus and inhibit ovulation (Imwalle et al., 2002). Feedlots commonly use MGA to suppress ovulation, which causes increased weight gain.

The feeding level of MGA is critical for the success of an estrus synchronization protocol. Melengestrol acetate can be delivered with grain, a protein carrier, top dressed on feed, or match mixed with a larger quantity of feed. If the animals do not receive the required amount they will prematurely exhibit estrus during the feeding period. Advantages to using MGA are ease of administration, lower cost compared to other estrus synchronization products, and its potential to induce estrus in prepubertal heifers.

Many estrus synchronization protocols utilize MGA; it can be used alone with natural service, in combination with GnRH and/or PG with the implementation of heat detection and AI or fixed-time AI (FTAI). This review will focus on the MGA-PG protocol in heifers. In this protocol, heifers are fed MGA for 14 d, given an injection of PG 19 d after MGA withdrawal and fixed-time AI 72 h following PG. At AI heifers are given an injection of GnRH (Figure 4).

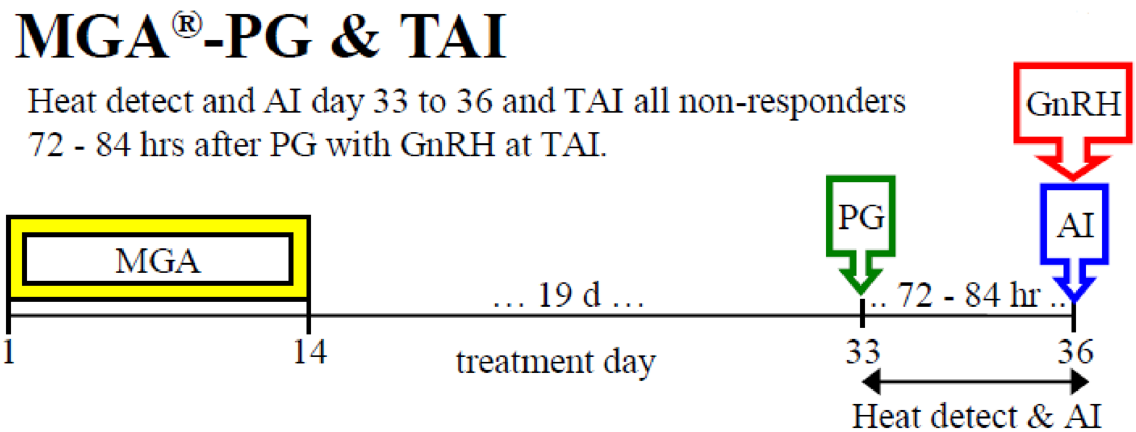

Figure 4. Melengestrol acetate-prostaglandin and timed artificial insemination (MGA-PG & TAI) protocol for beef heifers. MGA is administered orally at .5mg/hd/d for 14 d, 19 d after MGA withdraw an injection of prostaglandin $F_{2\alpha}$ (PG) is administered, 72 h following the PG injection heifers are given an injection of gonadotropin releasing hormone (GnRH) at time AI. (Adapted from Beef Reproductive Task Force)

This combination of feeding MGA for 14 d and waiting 19 d before PG injection has been developed after multiple phases of synchronization development (Figure 5). However, there was little benefit or sometimes a reduction in fertility, which could partly be contributed to the day of the estrous cycle for each heifer at treatment initiation (Figure 6). Heifers will exhibit estrus following the termination of feeding MGA, however fertility is reduced on this estrus. Low dose, high frequency pulses of LH can cause large persistent follicles to form on the ovary due to low progesterone supplementation (MGA). Follicle fertility is compromised due to altered hormone concentration and age of the follicle (Inskeep, 2004; Figure 7).

Phase	Period	Length of treatment	Dosage, mg/d	Combined with	Synchronization[a]	Fertility[b]
I	1963-1970	10-18 d	.5-1.0		Good[j]	Reduced[l]
II	1968-1974	18-32 d	.4-.5	E_2-17β[di]	Good	Reduced
				ECP[ei]	Good	Reduced
				hCG[fi]	Poor[k]	Reduced
				LH[gi]	Poor	Reduced
				PMSG[hi]	Poor	Reduced
				Oxytocin[i]	Poor	Reduced
III[c]	1972-1978					
IV	1974-1988	7 d	.5	$PGF_{2\alpha}$ d 7	Good	Reduced
		14 d	.5	$PGF_{2\alpha}$ 17 d after MGA	Good	Good[m]

[a]Response to treatment after withdrawal of MGA and during the synchronized period.
[b]Conception rates at first service after treatment withdrawal and during the synchronized period.
[c]Period coinciding with the development of prostaglandin $F_{2\alpha}$.
[d]Estradiol-17β.
[e]Estradiol cypionate.
[f]Human chorionic gonadotropin.
[g]Luteinizing hormone.
[h]Pregnant mare serum gonadotropin.
[i]Administered on d 8 to d 13 of treatment with MGA.
[j]More than 75% of the treated animals exhibited estrus during the synchronized period.
[k]Less than 75% of the treated animals exhibited estrus during the synchronized period.
[l]Conception rates were lower than those of controls inseminated at a spontaneous estrus.
[m]Compared only with Syncro-Mate-B ®.

Figure 5. Development of estrous cycle control utilizing melengestrol acetate (MGA). A summary table of the 4 phases of MGA estrous synchronization protocol development. (Adapted from Patterson et al., 1989)

Day of the estrous cycle[a]	Aggregate conception rates for day of the estrous cycle treatment began	
	SMB[b]	MGA + PGF[c]
0–5	10/21 (47%)	18/22 (82%)
6–11	12/26 (46%)	9/13 (69%)
12–16	9/24 (37%)	5/12 (42%)
17–21	5/14 (36%)	3/14 (21%)

[a]Day of the estrous cycle on which respective treatments began (estrus = d 0).
[b]SMB = Syncro-Mate-B® (from Brink and Kiracofe, 1988).
[c]MGA + PGF = 7 d MGA + Fenprostalene® d 7 (from Patterson et al., 1989).

Figure 6. Conception rates of heifers treated with Syncro-Mate-B or MGA-PG on different days of the estrous cycle. (Adapted from Patterson et al., 1989)

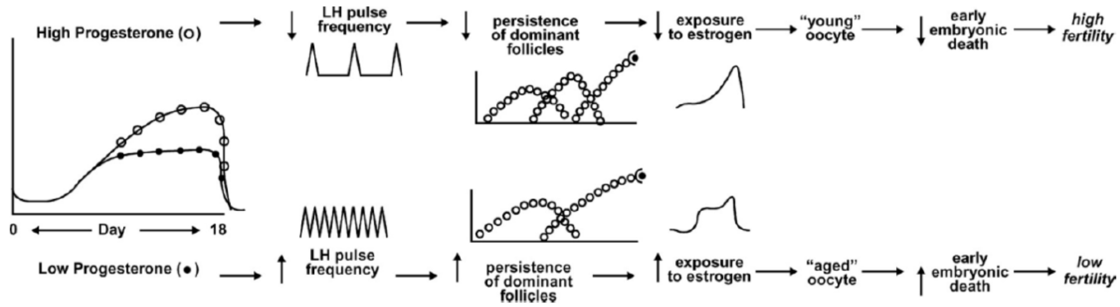

Figure 7. Effect of high (normal) and low dose (MGA) progesterone on LH frequency, occurrence of persistent follicles, estrogen exposure, oocyte quality, risk of early embryonic mortality and resulting fertility. (Adapted from Inskeep, 2004)

When utilizing the MGA-PG estrus synchronization protocol, heifers can be estrus detected with AI carried out 12 h later and heifers that do not show signs of estrus are subjected to timed AI, with an injection of GnRH given at AI or all heifers subjected to strict timed AI with an injection of GnRH. Larson et al. (1996) conducted 2 experiments to determine conception rates of heifers to time AI after MGA-PG, however this protocol was 17 d between MGA withdraw and PG injection. In the first experiment, heifers were subjected to timed AI at 72 h after PG regardless of estrus behavior, there was a tendency for this to increase the percentage of heifers that became pregnant to AI compared with heifers inseminated 12 h after they exhibited estrus behavior. In the second experiment, the number of heifers that conceived to AI was increased by mass inseminating all heifers that did not show signs of estrus by 72 h.

Lamb et al., (2000) performed a study where heifers were injected with PG on day 17 or 19 after MGA withdrawal. Heifers injected on d 19 had a shorter interval to estrus with 99% exhibiting estrus and inseminated by 72 h, compared with 74% in the 17 day after withdrawal injection group. In a study done by Johnson and Day (2004), they compared MGA-PG (19 day PG injection) protocol with estrus detection and AI (EA), time AI (TAI) only, and estrus detection AI and clean up AI (EAC). In this study, EA resulted in 63% pregnancy rate, TAI at 60 h after PG resulted in 46.6% pregnancy, and EAC with clean up AI at 80 h after PG resulted in 63.5%.

Although it has been shown MGA-PG works well for inducing puberty, synchronizing estrus, and resulting in acceptable pregnancy rates, there is another source of progesterone of interest to producers, the controlled internal drug release (CIDR).

Controlled Internal Drug Release

Another method of estrus synchronization in cattle is the use of a CIDR. The CIDR is a “T” shaped device inserted in the vagina of a cow or heifer and contains 1.38g of progesterone. This device can be somewhat costly to beef producers when compared with MGA, however CIDRs eliminate the need for cattle to be in a feedlot and/or provide bunk space as needed with MGA. Additionally, with MGA it is essential all females to be synchronized orally intake 0.5mg/hd/d for MGA to work properly. With the CIDR, there is a constant release of progesterone and no need to worry if cattle are receiving the proper amount. There is the chance a CIDR will come out of the vagina before the end of the treatment period; however, the retention rate has been about 97%, but this depends on each operation (Lamb and Larson, 2004).

There are many estrus synchronization protocols utilizing CIDRs and fixed time AI that produce consistent pregnancy rates in mature cows, particularly the Co-Synch + CIDR protocol. The Co-Synch + CIDR protocol starts with an injection of GnRH and CIDR placement on day 0, CIDR removal and a PG injection on d 7, followed by AI and a GnRH injection 54 h later (Figure 8). In cows, this protocol can yield FTAI pregnancy rates between 50 to 60 %. When utilizing the Co-Synch + CIDR protocol in heifers, Busch et al. (2007) found greater FTAI pregnancy rates were achieved when utilizing the CIDR Select protocol versus the Co-Synch + CIDR protocol (63 vs. 43%, respectively). The CIDR Select protocol requires a longer duration of progesterone administration, with the CIDR being in the vagina of the heifer for 14 d compared to 7 d.

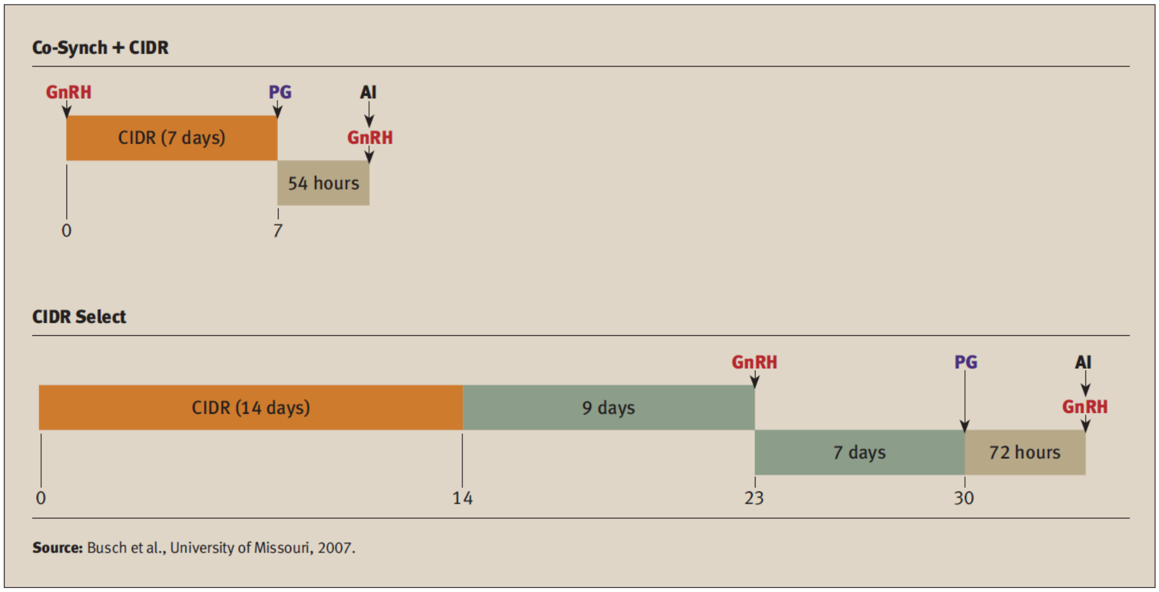

Figure 8. Co-Synch + CIDR and CIDR Select estrous synchronization protocols. The Co-Synch + CIDR starts with an injection of gonadotropin releasing hormone (GnRH) on d 0, along with the controlled internal drug release (CIDR) insert, on d 7 the CIDR is removed and an injection of prostaglandin $F_{2\alpha}$ (PG) is administered, AI occurs 54 h later with an injection of GnRH. The CIDR Select protocol starts with a CIDR insert on d 0 and is removed on d 14, 9 d following CIDR removal an injection of GnRH is administered, 7 d following an injection of PG is administered, and 72 h later GnRH is administered at AI. (Adapted from Busch et al., 2007)

Additionally in the study by Busch et al. (2007), the CIDR select protocol demonstrated greater and a more synchronized estrus response prior to FTAI and the estrus following. Leitman et al. (2009) compared 4 estrus synchronization protocols utilizing a CIDR for 14 d, with or without GnRH. All treatments received an injection of

PG on d 30, heat detected, and AI approximately 12 h later. There were no differences in estrous response among treatments, conception rates to AI were consistently 60%, and final pregnancy rates were similar (83 to 92%).

Kojima et al. (2004) compared MGA-Select and CIDR-Select protocols to evaluate reproductive efficacy. Both protocols utilize MGA or CIDR for 14 d, an injection of GnRH 12 d after MGA withdraw, 9 d after CIDR removal and PG injected 7 d following GnRH injection. In this study, estrus synchronization response did not differ, however AI pregnancy rate was greater (63 v. 47%, CIDR v. MGA, respectively) for heifers synchronized with the CIDR-Select protocol. By the end of the study similar pregnancy rates were observed between estrus synchronization protocols.

Conclusions

Current literature provides us great insight on how to influence reproduction utilizing estrous synchronization and AI, as well as manage nutrition to develop replacement heifers that are fertile prior to the first breeding season. However, many of the industry guidelines used in management practices today, are based on study results performed several decades ago. The beef industry has been changing rapidly due to advances in genetic technologies (EPDs and genomics), the increase in composite cow herds, and the changes in cattle feeding due to increased feedstuff prices driving producers to look for different options. Today’s cow herds are different from cow herds a couple decades ago and it is important to continue to research important topics related to reproductive development as changes occur.

Therefore, the following 3 research chapters will address fertility in beef heifers. Chapter II will address the industry standard of developing heifers to have at least 2 estrous cycles prior to the first breeding season. This standard was established mainly by two studies from 1971 and 1987. Chapter III will evaluate the effect of BW gain at different time periods during development on puberty and pregnancy rates. From the literature, it is known BW gain plays an important role in puberty attainment and pregnancy establishment. There is no clear evidence if BW gain during specific time points, throughout development, are critical for reproductive success. And finally, Chapter IV will evaluate the reproductive efficacy of 2 estrus synchronization protocols commonly used in beef heifers. The research is limited to these 2 protocols when utilizing timed AI, which is important in reducing labor needs when implementing estrus synchronization and AI.

Objectives

- Determine if the fertility of the first estrus differs from the fertility of subsequent estrus.
- Evaluate the effect of ADG at different development periods on the onset of puberty prior to the breeding season and pregnancy rates.
- Compare the reproductive efficacy and economics of 2, 14-d progestin estrus synchronization protocols in beef heifers.

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CHAPTER II:

Effect of pubertal status and number of estrous cycles prior to the breeding season on pregnancy rate in beef heifers

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Abstract

Three experiments were conducted to evaluate whether pubertal status prior to breeding influences pregnancy rate in beef heifers. Records were collected at West Central Research and Extension Center, North Platte, Neb., from 2002 to 2011 (Exp. 1; n = 1,005) and Gudmundsen Sandhills Laboratory, Whitman, Neb., from 1997 to 2011 (Exp. 2; n = 1,253). Heifers in Exp. 1 and 2 were classified as either being pubertal or non-pubertal at the start of breeding. In Exp. 3, (n = 156) heifers were classified by number of estrous cycles (0, 1, 2, 3, or ≥ 4) exhibited prior to breeding. In Exp. 1 and 2, pubertal heifers were heavier ($P \leq 0.04$) and older ($P < 0.07$) at start of breeding and had a greater ($P < 0.01$) overall pregnancy rate (94 vs. 88 ± 2%, 90 vs. 82 ± 2% in Exp. 1 and 2, respectively) than non-pubertal heifers. Pubertal heifers also tended ($P = 0.08$) to have greater AI pregnancy rate (62 vs. 56 ± 4%, Exp. 1), produced more calves within the first 21 d of calving ($P < 0.01$), and weaned older ($P = 0.05$), heavier ($P < 0.01$) calves than heifers that had not reached puberty (Exp. 2). In Exp. 3, heifers pubertal prior to the breeding season had greater (85 ± 8%, $P = 0.05$) pregnancy rates (68 ± 8%) than non-pubertal heifers and pregnancy rate tended ($P = 0.15$) to be influenced by the number of estrous cycles (68, 81, 91, 93, and 82 ± 9% for 0, 1, 2, 3, or ≥4 estrous cycles, respectively). Second season pregnancy rate was greater for heifers reaching puberty prior to first breeding (97 vs. 80 ± 7%, $P < 0.01$) and was influenced ($P = 0.03$) by the number of estrous cycles, where heifers having ≥ 2 estrous cycles had greater pregnancy rate (80, 87, 100, 97, and 98 ± 8% for 0, 1, 2, 3, or ≥ 4 estrous cycles, respectively) than non-pubertal heifers. Pregnancy rate was greater for heifers achieving puberty prior to breeding, which was influenced by age and BW. However, earlier onset of puberty did not significantly improve first pregnancy rates.

Introduction

Replacement heifer development can significantly impact the profitability of a beef cattle operation. It is imperative replacement females be developed economically to have their first calf at 2 yr of age. In order for this to occur, puberty must be attained by approximately 13 to 15 mo of age. Heifers that conceive early in the breeding season calve earlier and wean heavier calves, increasing longevity and productivity within the herd (Short and Bellows, 1971; Lesmeister et al., 1973; Funston et al., 2012a). Breeding heifers at 13 to 15 mo of age may be a disadvantage to later maturing heifers, as pregnancy rates have been correlated with the percentage of heifers that reach puberty before or early in the breeding season (Short and Bellows, 1971; Perry et al., 1991). Byerley et al. (1987) demonstrated heifers inseminated on pubertal estrus had a decreased pregnancy rate compared with heifers inseminated on their third estrus. However, heifers inseminated on pubertal estrus were inseminated at an earlier date than heifers inseminated on the third estrus. Therefore, heifers inseminated on the pubertal estrus were younger and weighed less at breeding.

Recent research has demonstrated heifers developed to a lower pre-breeding BW achieved acceptable pregnancy rates (Funston et al., 2012b) and in a recent review on heifer development and lifetime productivity (Endecott et al., 2013), it was hypothesized that genetic selection with EPD implementation for traits such as growth, milk, carcass characteristics, and scrotal circumference may have contributed to changes in beef heifer reproductive performance over time.

Therefore, the objectives of this study were to determine the effect of pubertal status and the number of estrous cycles prior to breeding on pregnancy rates in beef heifers.

Materials and Methods

All animal procedures and facilities were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Data were collected from the West Central Research and Extension Center (**WCREC**), North Platte (2002 to 2011; n = 1,005, **Exp. 1**), and Gudmundsen Sandhills Laboratory (**GSL**), Whitman (1997 to 2011; n = 1,253, **Exp. 2**; n = 156, **Exp. 3**). Heifers at WCREC were Angus-based and synchronized with a melengestrol acetate-$PGF_{2\alpha}$ protocol (Funston and Larson, 2011) prior to AI. Approximately 10 d following AI, heifers were exposed to fertile bulls at a bull to heifer ratio of 1:50 for 60 d. Conception to AI was determined 45 d after AI by transrectal ultrasonography and final pregnancy rate was determined via transrectal ultrasonography 45 d following removal of bulls.

Data from GSL were collected on a spring calving herd of composite Red Angus × Simmental females. Heifers were exposed to bulls for 45 d at a bull to heifer ratio of 1:25. A single injection of $PGF_{2\alpha}$ (Prostamate, Teva Animal Health Inc., St. Joseph, MO, or Lutalyse, Pfizer Animal Health, New York, NY) was administered i.m. to heifers 108 h after placement with bulls. Pregnancy determination was performed via transrectal ultrasonography approximately 45 d after the breeding season.

Pubertal status was determined by evaluating progesterone concentration in 2 blood samples collected via coccygeal venipuncture 10 d apart prior to the breeding season for Exp. 1 and 2. The number of estrous cycles prior to the breeding season in Exp. 3 was determined via serial blood collection every 10 d beginning in early January of each year until the beginning of the breeding season (late May). Heifers in Exp. 3 were further classified as non-pubertal or pubertal (0 vs. ≥ 1 estrous cycle) and as having exhibited 1 estrous cycle or greater than or equal to 2 estrous cycles (1 vs. ≥ 2), excluding heifers that had not reached puberty prior to breeding, to evaluate effects on pregnancy rate. Blood samples were stored at 4°C for serum separation by centrifugation (2,500 × g for 20 min at 4°C) within 24 h. Serum samples were stored at -20°C for subsequent analysis. Serum progesterone concentrations were determined by direct solid-phase RIA (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) without extraction as described by Melvin et al. (1999). Intra- and interassay CV were 4.2 and 2.8%, respectively. Progesterone concentration > 1 ng/ mL was interpreted to indicate ovarian luteal activity.

Statistical analysis.

Data were analyzed using PROC GLIMMIX of SAS 9.2 (SAS Inst., Inc., Cary, NC). Means were separated using LSD. Effects of pubertal status or number of estrous cycles were considered to be significant when $P \leq 0.05$, a tendency when $P \leq 0.10$, or a trend when $P \leq 0.15$.

Results and Discussion

Exp. 1

Date of birth, BW, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding are presented in Table 1. Julian birth date was similar ($P = 0.12$) for heifers that were pubertal or non-pubertal. Pubertal heifers had greater ($P < 0.01$) BW compared with non-pubertal heifers from weaning through final pregnancy diagnosis. Weaning to final pregnancy diagnosis ADG was similar ($P = 0.62$; 0.54 vs. 0.53 ± 0.05 kg/d for non-pubertal and pubertal, respectively) for pubertal and non-pubertal heifers providing evidence that differences in post-weaning BW were likely due to greater pre-weaning ADG for heifers that reached puberty prior to breeding. These results are consistent with previous research indicating pre-weaning growth exerts a greater influence on puberty than post-weaning growth (Patterson et al., 1992; Roberts et al., 2009). This also agrees with previous research reporting ADG prior to breeding has minimal impact on pubertal status and pregnancy rates (Funston and Deutscher, 2004; Martin et al., 2007; Funston and Larson, 2011; Larson et al., 2011).

Heifers that were pubertal prior to breeding tended ($P = 0.08$) to have greater AI pregnancy rate (62 vs. 56 ± 4%) and greater ($P < 0.01$) overall pregnancy rate (94 vs. 88 ± 2%) compared with non-pubertal heifers. Days to calving was decreased ($P < 0.01$) for pubertal vs. non-pubertal heifers, however, calf birth BW did not differ ($P = 0.92$, 34 ± .7 kg).

Exp. 2

Date of birth, BW, ADG, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding are presented in Table 2. Heifers that were pubertal prior to breeding were born approximately 4 d earlier ($P < 0.01$) than non-pubertal heifers.

Heifer birth BW did not differ ($P = 0.28$) between groups. However, pubertal heifers had greater ($P < 0.01$) weaning and pre-breeding BW, and tended ($P = 0.08$) to be heavier at pregnancy diagnosis than non-pubertal heifers. Heifers that were pubertal prior to breeding had greater ($P < 0.01$) ADG from birth to weaning, similar to what is being hypothesized for Exp. 1. Heifers that did not reach puberty prior to breeding tended ($P = 0.09$) to have greater ADG from weaning to pre-breeding and had greater ($P < 0.01$) ADG from breeding to pregnancy diagnosis. The greater ADG from weaning to pregnancy diagnosis by non-pubertal heifers resulted in a similar ($P = 0.41$) BW at pre-calving.

Pregnancy rate was greater ($P < 0.01$) for pubertal heifers vs. non-pubertal heifers (90 vs. 84 ± 2%, respectively). A greater ($P < 0.01$) proportion of pubertal heifers calved within the first 21d of the calving season compared with heifers classified as non-pubertal prior to breeding. Date of calving was 5 d earlier for heifers that were pubertal prior to breeding and their calves were heavier ($P < 0.01$) at birth and were heavier and older ($P < 0.05$) at weaning than calves from heifers that were not pubertal prior to breeding. At weaning, there was no difference ($P > 0.90$) in BW (317 ± 8.4 kg) and BCS (5.1 ± 0.1) between first calf heifers classified as pubertal or non-pubertal before start of breeding as heifers. Second season pregnancy rate was also similar ($P = 0.65$) between groups.

Exp. 3

Date of birth, BW, ADG, pregnancy rate, and first calf characteristics are presented in Table 3 for heifers classified by number of estrous cycles prior to the breeding season. Heifers had similar ($P = 0.34$) birth BW regardless of number of estrous cycles prior to breeding. There was a trend ($P = 0.12$) for heifers that had 3 estrous cycles prior to the breeding season to be born earlier and a tendency ($P = 0.10$) to have greater weaning BW compared with heifers that exhibited estrus ≤ 2 and ≥ 4 times.

Heifers exhibiting ≥ 4 estrous cycles were younger ($P < 0.01$; 409, 394, 379, 324 ± 6.3 d, for 1, 2, 3, and ≥ 4 estrous cycle groups, respectively) and had reduced ($P < 0.01$) BW at puberty than heifers exhibiting estrus ≤ 3 times. Heifers that exhibited ≤ 3 estrous cycles had similar ($P \geq 0.92$) BW at puberty. Birth to weaning ADG was similar ($P = 0.27$); however, heifers that had ≥ 4 cycles had lower ($P < 0.01$) weaning to puberty ADG compared with heifers that exhibited estrus ≤ 3 times. Heifer BW was similar ($P \geq 0.16$) at pre-breeding and pregnancy diagnosis.

There was a trend ($P = 0.15$) for pregnancy rate to increase with the number of estrous cycles exhibited prior to breeding. Heifers that were pubertal prior to breeding had greater ($P = 0.05$; 85 vs. 68 ± 8%) pregnancy rate than non-pubertal heifers. Pregnancy rate did not differ for heifers having 1 estrous cycle compared with heifers having ≥ 2 estrous cycles prior to breeding ($P = 0.68$; 81 vs. 85 ± 9% for 1 and ≥ 2, respectively). In contrast, Byerley et al. (1987) reported pregnancy rate was decreased 21 percentage points for heifers inseminated at pubertal estrus compared with third estrus. In the current study, heifers were placed with bulls or AI on a common date resulting in similar age at breeding, whereas date of insemination in Byerley et al. (1987) was earlier for heifers at pubertal estrus compared with heifers inseminated on third estrus, resulting in heifers inseminated on first estrus being approximately 50 d younger at breeding.

Pre-calving BW tended to be greater for heifers exhibiting ≥ 3 estrous cycles than those exhibiting estrus ≤ 2 times prior to breeding. The proportion of heifers that calved within the first 21 d, calf birth date, and calf birth BW did not differ ($P \geq 0.20$) among estrous cycle classifications prior to breeding.

Heifers that were pubertal prior to the first breeding season had a greater ($P < 0.01$) second season pregnancy rate than heifers that were non-pubertal prior to the first breeding season (97 vs. 80 ± 7%). Second season pregnancy rate was greater ($P = 0.03$) for heifers having ≥ 2 estrous cycle prior to the first breeding season than heifers having ≤ 1 estrous cycle, however heifers that had 0 or 1 estrous cycle had similar ($P = 0.81$) second season pregnancy rates (80, 87, 100, 97, and 98 ± 8% for 0, 1, 2, 3, and ≥ 4 estrous cycle groups, respectively). Heifers with ≥ 2 estrous cycles prior to the first breeding season also tended ($P = 0.08$) to have greater second season pregnancy rate compared with heifers that had 1 estrous cycle (98 vs. 88 ± 6% for ≥ 2 and 1 estrous cycles, respectively).

Implications

In most beef operations heifers are inseminated to calve at approximately 24 mo of age. This requires heifers to attain puberty and conceive by 15 mo of age. It has been previously suggested that heifers should reach puberty 1 to 3 mo prior to the breeding season to obtain greater fertility. Results from this study suggest if a heifer attains puberty prior to the breeding season, acceptable pregnancy rates can be achieved regardless of the number of estrous cycles experienced prior to breeding. However, additional research is needed to further substantiate the potential impacts pubertal status prior to the start of breeding may have on second season pregnancy rates.

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Table 1. Birth date, BW, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding. (Exp. 1)

	Pubertal	Non-Pubertal	SE	*P*-value
N	695	310		
Julian birth date[1], d	78.9	81.9	1.5	0.12
Weaning BW, kg	270	232	4.3	<0.01
AI BW, kg	357	348	12.1	<0.01
AI pregnancy rate, %	61.9	55.5	3.7	0.08
Overall pregnancy diagnosis BW, kg	423	416	8.3	<0.01
Overall pregnancy rate, %	94.2	87.7	1.9	<0.01
Days to calving[2], d	284	288	2.0	<0.01
Calve within first 21d[3], %	77.8	66.2	5.1	<0.01

[1]Birth date was known for only a subset of heifers (n = 360).
[2]Days from start of breeding season to calving.
[3]Calved within the first 21 d of the calving season, d 1 refers to the day the first calf is born.

Table 2. Birth date, BW, ADG, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding. (Exp. 2)

	Pubertal	Non-Pubertal	SE	*P*-value
N	752	491		
Julian birth date, d	83.9	87.8	4.8	<0.01
Born first 21d[1], %	63.8	49.7	5.9	<0.01
Birth BW, kg	34.8	35.2	0.64	0.28
Weaning BW, kg	209	202	3.0	<0.01
Birth to weaning ADG, kg	0.79	0.77	0.5	<0.01
Pre-breeding age, d	428	424	2.9	<0.01
Pre-breed BW, kg	302	294	4.4	<0.01
Pre-breed ADG, kg	0.45	0.46	0.03	0.09
Pregnancy diagnosis BW, kg	368	365	4.4	0.08
Breeding to pregnancy diagnosis ADG, kg	0.64	0.68	0.05	<0.01
Pregnancy rate, %	90.0	84.2	2.0	<0.01
Pre-calving BW, kg	423	421	7.1	0.41
Calve within first 21d[2], %	79.1	67.0	4.3	<0.01
Calf Julian birth date, d	75	80	4.9	<0.01
Calf birth BW, kg	33	32	0.5	<0.01
Calf weaning BW, kg	187	177	5.4	<0.01
Calf weaning age, d	181	177	3.8	0.05
Cow BW at weaning, kg	417	417	8.4	0.99
Cow BCS at weaning	5.1	5.1	0.1	0.91
Second pregnancy rate , %	89.8	91.2	3.1	0.65

[1]Born within the first 21 d of calving season, d 1 is the day the first calf is born.
[2]Calved within the first 21 d of the calving season, d 1 is the day the first calf is born.

Table 3. Birth date, BW, ADG, pregnancy rate, and first calf characteristics of heifers classified by number of estrous cycles prior to breeding. (Exp. 3)

	0	1	2	3	≥4	SE	*P*-value
n	25	16	22	27	66	156	
Julian birth date, d	85.3	85.9	85.8	78.2	84.0	3.1	0.12
Born first 21d[1], %	67.8	80.8	73.1	93.0	78.7	9.3	0.24
Birth BW, kg	35.7	33.8	34.2	36.3	35.3	1.3	0.34
Weaning BW, kg	222	224	230	238	229	7.7	0.10
Age at puberty, d	-	409[a]	394[a]	379[b]	324[c]	6.3	<0.01
Puberty BW, kg	-	316[a]	323[a]	315[a]	260[b]	13.7	<0.01
Wean to puberty ADG, kg/d	-	1.08[a]	1.09[a]	1.09[a]	0.61[b]	1.09	<0.01
Pre-breed BW, kg	376	383	392	406	385	17.4	0.16
Pregnancy diagnosis BW, kg	362	365	366	380	364	13.2	0.27
Pregnancy rate, %	68.0	81.3	90.9	92.6	81.8	9.4	0.15
Wean to pregnancy diagnosis ADG, kg	0.48	0.49	0.48	0.49	0.47	0.04	0.79
Puberty to pregnancy diagnosis ADG, kg	-	0.52[ab]	0.38[c]	0.49[b]	0.57[a]	0.34	<0.01
Pre-calving BW, kg	426	426	441	455	435	16.1	0.10
Calve within first 21d[2], %	65.3	83.6	87.6	82.7	75.1	14.2	0.47
Calf Julian birth date, d	72.5	66.9	63.4	67.8	68.8	4.5	0.20
Calf birth BW, kg	30.7	30.5	31.2	31.9	31.5	1.4	0.78
Second pre-breed BW, kg	376	383	392	406	385	17.5	0.16
Second pregnancy rate, %	79.5[b]	87.2[ab]	100.0[a]	97.0[a]	97.9[a]	8.0	0.03

[a-c]Means without a common superscript differ ($P \leq 0.05$).
[1]Born within the first 21 d of calving season, d 1 is the day the first calf is born.
[2]Calved within the first 21 d of the calving season, d 1 is the day the first calf is born.

CHAPTER III:

Effect of average daily gain (ADG) on pubertal status and pregnancy in beef heifers

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Abstract

Beef heifers at 2 locations were analyzed to evaluate the effect of ADG on pubertal status and pregnancy rates. Records were collected at Gudmundsen Sandhills Laboratory, Whitman, NE from 1997 to 2011 (GSL; n = 1,253) and the West Central Research and Extension Center, North Platte, NE from 2002 to 2011 (WCREC; n = 1,005). Body weights were measured throughout development and ADG calculated. All heifers were classified as either being pubertal or non-pubertal at the start of breeding. Pubertal status was affected by ADG from weaning to breeding ($P < 0.01$) for GSL and WCREC and birth to weaning ADG ($P < 0.01$) for GSL heifers. An increase in ADG increased the odds of attaining puberty prior to breeding. Heifers from GSL and WCREC that were pubertal prior to the breeding season had greater ($P < 0.05$) odds of becoming pregnant by the end of the breeding season. There was an interaction ($P < 0.01$) of ADG from breeding to final pregnancy ultrasound for GSL heifers. As ADG increased for pubertal heifers, odds of pregnancy increased, however non-pubertal heifers had lower odds of pregnancy as ADG increased. Regardless of pubertal status WCREC heifers tended ($P = 0.06$) to have increased odds of AI pregnancy with increased ADG from

breeding (AI) to AI pregnancy ultrasound. Additionally, non-pubertal heifers tended (P = 0.06) to have increased odds of final pregnancy as ADG increased from breeding (AI) to final pregnancy ultrasound. Furthermore, increased ADG from AI pregnancy ultrasound to final pregnancy ultrasound tended (P = 0.07) to have a positive effect on the odds of final pregnancy.

Introduction

Age at puberty and fertility can be greatly affected by nutrition. Little attention has been focused on the effect of preweaning nutrition in regards to heifer reproduction. The studies that have focused on this period of development found BW gain to have a more consistent influence on puberty attainment than postweaning gain (Wiltbank et al., 1966; Roberts et al., 2009). Puberty has been found to spontaneously occur prior to 10 months of age when growth rate around weaning was increased (Wehrman et al., 1996). (Gasser, 2013) has reported greater incidence of precocious puberty when heifers were weaned early and fed a high concentrate diet.

More research focus has been on postweaning heifer development. Heifers of similar breed composition can attain puberty several months of part if fed different diets (Wiltbank et al., 1969; Short and Bellows, 1971). Studies have shown that heifers raised on a low energy diet reached puberty later and had lower pregnancy rates in the first breeding season compared with heifers developed on a high energy diet (Short and Bellows, 1971; Hall et al., 1995). In the same experiment by (Short and Bellows, 1971), heifers that exhibited medium (0.45 kg/d) and high (0.68 kg/d) ADG reached puberty earlier, 60% of these heifers conceived in the first 20 d of the breeding season, and had greater overall conception rates compared to low-gain heifers (0.23 kg/d).

Research and experience has set heifer “target weight” at 65% of mature BW (Patterson et al., 1992) at breeding. However, there has been some debate if this is the ideal target weight for beef heifers. Heifers have been developed to 53 to 55% of mature BW without compromised pregnancy rates and reduced costs. Additionally, dystocia, rebreeding rates, calf production traits, and longevity were similar in heifers developed to the lighter target weight compared to 60-65% of mature BW (Funston and Deutscher, 2004; Martin et al., 2008; Larson et al., 2010). The earlier studies that set the target weight were conducted in the early 1970s when there were more purebred cattle. Current studies, where heifers were developed to a lighter target weight utilized cross-bred/composite heifers, therefore purebred heifers may require being developed to a heavier target weight.

Much research has been focused on developing heifers to attain puberty in time to have multiple estrous cycles prior to breeding to ensure greater fertility during the first breeding season (Short and Bellows, 1971; Byerley et al., 1987). However, once the breeding season starts nutrition should continue to be the focus of management. Heifers developed in a drylot from weaning to breeding and moved to spring grass after breeding lost 16.8 kg from AI to pregnancy determination and had reduced pregnancy rates compared with heifers that were moved to grass and supplemented with 2.2 kg of distillers grains (Perry, 2012). Therefore, it is important to focus on plane of nutrition following breeding so fertility is not negatively influenced.

The objectives of this study were to determine how average daily gain prior to breeding influenced pubertal status and how ADG from birth through pregnancy diagnosis affected pregnancy rates.

Materials and Methods

All animal procedures and facilities were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Data were collected from the Gudmundsen Sandhills Laboratory (**GSL**), Whitman (1997 to 2011; n = 1,253, **GSL**) and the West Central Research and Extension Center, North Platte (2002 to 2011; n = 1,005, **WCREC**).

Heifer BW and date were recorded at GSL at birth, weaning, breeding, and pregnancy ultrasound each year. Average daily gains were calculated by dividing the 2 BW of interest divided by the number of days between weights, to get pounds of gain per day. The ADG categories for GSL are birth to weaning, weaning to breeding and breeding to pregnancy ultrasound. Similarly, at WCREC BW were taken at arrival (weaning), one month prior to breeding (April), breeding (May), AI pregnancy ultrasound, and final pregnancy ultrasound. Again, dates that BW were taken were also recorded. From this heifer ADG was calculated for weaning to breeding (AI), one month prior to breeding (AI), breeding to AI pregnancy ultrasound, breeding to final pregnancy ultrasound, and AI pregnancy ultrasound to final pregnancy ultrasound.

Heifers at WCREC were Angus-based and synchronized with a melengestrol acetate-$PGF_{2\alpha}$ protocol (Funston and Larson, 2011) prior to AI. Approximately 10 d following AI, heifers were exposed to fertile bulls at a bull to heifer ratio of 1:50 for 60 d.

Conception to AI was determined 45 d after AI by transrectal ultrasonography and final pregnancy rate was determined via transrectal ultrasonography 45 d following removal of bulls.

Data from GSL were collected on a spring calving herd of composite Red Angus × Simmental females. Heifers were exposed to bulls for 45 d at a bull to heifer ratio of 1:25. A single injection of $PGF_{2\alpha}$ (Prostamate, Teva Animal Health Inc., St. Joseph, MO, or Lutalyse, Pfizer Animal Health, New York, NY) was administered i.m. to heifers 108 h after placement with bulls. Pregnancy determination was performed via transrectal ultrasonography approximately 45 d after the breeding season.

Pubertal status was determined by evaluating progesterone concentration in 2 blood samples collected via coccygeal venipuncture 10 d apart prior to the breeding season. Blood samples were stored at 4°C for serum separation by centrifugation (2,500 × g for 20 min at 4°C) within 24 h. Serum samples were stored at −20°C for subsequent analysis. Serum progesterone concentrations were determined by direct solid-phase RIA (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) without extraction as described by Melvin et al. (1999). Intra- and interassay CV were 4.2 and 2.8%, respectively. Progesterone concentration > 1 ng/ mL was interpreted to indicate ovarian luteal activity.

Statistical analysis.

Data were analyzed using PROC GLIMMIX of SAS 9.3 (SAS Inst., Inc., Cary, NC). The effect of ADG on pubertal status was evaluated. This model included pubertal status as the response variable (binomial), the fixed effect ADG (ex: breeding to weaning

ADG) and random variable was year/treatment. The effect of ADG on pregnancy rates was also evaluated. This model included pregnancy status as the response variable (binomial), fixed effects were pubertal status, ADG (ex: birth to weaning), and the interaction of pubertal status and ADG. If there was no significant interaction ($P > 0.05$), the interaction was dropped from the model and only main effects will be reported. Data are considered significant at $P \leq 0.05$.

Odds ratios were calculated to obtain information about the attainment of puberty or the chance of pregnancy for different levels of ADG during different time periods of heifer development. The odds ratio is the probability of one outcome (attainment of puberty) relative to another (no attainment of puberty). The odds ratio of 2 levels of a fixed effect equal to 1 indicates no difference between the 2 levels.

Average daily gain for each time point included every 0.25 lbs based on 2 standard deviations from the mean. Odds for pubertal status or pregnancy status for ADG at each 0.25 lb increment was compared to the mean ADG for the time point of interest to get an odds ratio (**OR**).

Results and Discussion

Gudmundsen Sandhills Laboratory (GSL)

Pubertal status was affected by ADG from birth to weaning ($P < 0.01$; Table 1) and weaning to breeding ($P < 0.01$; Table 2). As ADG from birth to weaning increased the odds of becoming pubertal also increased (Table 1). This is also evidenced by the OR, from birth to weaning all ADGs are compared with the mean ADG, which is 1.75 lb/d; therefore, those heifers with an ADG between 1.0 and 1.50 had a lesser odds of becoming pubertal than heifers gaining 1.75 lb/d. Furthermore, heifers that gained between 2 and 2.45 lb/d from birth to weaning had a greater odds of becoming pubertal than heifers that gained 1.75 lb/d. These results confirm those of Wiltbank et al., 1966 and Roberts et al., 2009, where BW gain prior to weaning had consistent influence on pubertal status. Additionally, (Arije and Wiltbank, 1971) found greater BW gain from birth to weaning is negatively correlated with age at puberty.

Similar results were observed when ADG from weaning to breeding was evaluated for GSL heifers (Table 2). No differences were observed in the odds of becoming pubertal when heifers gained 0.25 to 0.50 lb/d, however the odds ratio of those heifers compared to heifers gaining 0.91 lb/d (mean) had lower odds of becoming pubertal. Furthermore, heifers that gained 1.0 to 1.5 lb/d had greater odds of becoming pubertal compared to heifers that gained 0.91 lb/d. This agrees with previous research in which heifers that gained more BW postweaning reached puberty at an earlier age than heifers that gained BW at a slower rate (Short and Bellows, 1971; Hall et al., 1995).

Average daily gain was evaluated for an effect on pregnancy rates. When evaluating ADG from birth to weaning, there was no interaction of pubertal status and ADG (P = 0.63) or main effect of ADG (P = 0.40); however there was an effect of pubertal status (P < 0.01, Table 3). Heifers cycling prior to the breeding season had greater odds of becoming pregnant during the breeding season (OR 1.94). Average daily gain from weaning to breeding had a similar effect on pregnancy rates. There was no interaction between weaning to breeding ADG and pubertal status (P = 0.29), no main effect of weaning to breeding ADG (P = 0.82); however there was an effect of pubertal status ($P < 0.01$; Table 3). Again, heifers pubertal prior to breeding had greater odds of becoming pregnant during the breeding season (OR 2.01).

Additionally, ADG from breeding to pregnancy ultrasound was evaluated for an effect on pregnancy rates. There was an interaction of pubertal status and ADG from breeding to pregnancy ultrasound on pregnancy rates ($P < 0.01$, Figure 1). Heifers that were pubertal prior to breeding had greater odds of becoming pregnant as ADG from breeding to pregnancy ultrasound increased, however those heifer not reaching puberty prior to breeding had decreased odds of becoming pregnant as ADG from breeding to pregnancy ultrasound increased (Figure 1). According to a review by (Perry, 2012) heifers that maintain and/or gain BW after breeding have a higher conception rate than those that lose BW. Perhaps those heifers that were non-pubertal prior to breeding are larger framed, later maturing, or exhibiting a fleshing effect where increased BW does not increase the odds of pregnancy.

West Central Research and Extension Center

Heifers were evaluated for the effect of ADG from weaning to breeding and one month prior to breeding (April to May) on pubertal status. Results generated show both weaning to breeding and one month prior breeding ADG significantly ($P < 0.01$; Table 4) affect heifer pubertal status. The odds of a heifer attaining puberty decreased ($P < 0.01$) as ADG increased from weaning to breeding. Similar results were observed when evaluating ADG one month prior to breeding with the odds of puberty attainment decreasing ($P < 0.01$; Table 5) with increasing ADG.

The effect of ADG on AI and natural service (NS) pregnancy rates was evaluated in the WCREC heifers. Weaning to breeding ADG was evaluated for its effects on AI pregnancy rates. No significant ($P = 0.23$) interaction was observed of pubertal status by ADG, however there was a main effect of pubertal status ($P < 0.01$) and a tendency ($P = 0.09$) of ADG to have an effect on AI pregnancy. The odds of becoming AI pregnant was 40.14 times greater if the heifer was pubertal prior to breeding.

When evaluating ADG one month prior to breeding for an effect on AI pregnancy, ADG did not affect ($P = 0.63$) pubertal status, the main effect of ADG was not significant ($P = 0.30$): however pubertal status affected AI pregnancy. The odds of AI pregnancy were 38.36 times greater if the heifer was pubertal prior to the breeding season. Additionally, the effect of ADG from AI to AI pregnancy ultrasound was evaluated for an effect on AI pregnancy rate. There was a significant ($P = 0.04$; Figure 2) interaction of pubertal status and ADG on AI pregnancy rates. Heifers that were pubertal prior to breeding had a 7.68 greater odds of becoming AI pregnant than heifers not pubertal prior to breeding. Increased BW gain increased the odds of AI pregnancy for heifers that were pubertal and non-pubertal prior to breeding, however pubertal heifers had much greater odds of pregnancy than non-pubertal heifers.

Furthermore, ADG at multiple time points was evaluated for an effect on final pregnancy rates. Weaning to breeding ADG by pubertal status interaction was not significant ($P = 0.36$), nor was there a significant ($P = 0.85$) effect of ADG. A tendency ($P < 0.06$) for pubertal status to affect final pregnancy rates was observed. Heifers that were pubertal prior to the beginning of the breeding season had 1.86 greater odds of becoming pregnant by the end of the breeding season. There was no significant interaction (*P* = 0.22) of ADG during the month prior to breeding by pubertal status. There was no significant (*P* = 0.88) main effect of ADG, however pubertal status (*P* < 0.01, OR 1.96) affected final pregnancy rates. Average daily gain from AI to AI pregnancy ultrasound and pubertal status interaction tended (*P* = 0.06) to influence final pregnancy rates (Figure 3). Increased BW gain for heifers that were pubertal prior to breeding did not have a great influence on increasing the odds of pregnancy; however, in heifers that had not reached puberty prior to breeding, odds for pregnancy increased as ADG increased during this period. There was no significant (*P* = 0.85) main effect of ADG, however pubertal status significantly (*P* < 0.01, OR 2.19) affected final pregnancy rates.

Average daily gain from start of breeding to final pregnancy ultrasound was evaluated for its effects on final pregnancy rates. There was a tendency (*P* = 0.06) for the interaction of pubertal status and ADG to have an effect on final pregnancy rates (Figure 4). Increased BW gain for heifers not pubertal prior to the start of the breeding season increased their odds of pregnancy; however, odds of pregnancy were not influenced by rate of BW gain in heifers that attained puberty prior to breeding. There was no significant (*P* = 0.70) main effect of ADG, however pubertal status had a significant effect (*P* < 0.01, OR 2.87).

Finally, ADG from AI ultrasound to final pregnancy ultrasound was evaluated for an effect on final pregnancy rates. There was no significant (*P* = 0.45) interaction of pubertal status and ADG on final pregnancy rates, however there was a tendency (*P* = 0.07, Table 6) for ADG to have an effect on final pregnancy rates. Additionally, pubertal status had a significant (*P* = 0.01, OR 1.95) effect on final pregnancy rates.

Body weight gain prior to breeding increased the odds of a heifer to become pubertal prior to the breeding season for GSL heifers. Heifers from WCREC had a different response to increased ADG from weaning to breeding, as BW gain increased the odds of attaining puberty prior to breeding decreased. This may be due to the differences in breed composition and/or frame score of the animals from WCREC vs. GSL. Larger framed, later maturing cattle may still be putting the excess BW gain into growth rather than reproduction prior to breeding at WCREC.

Average daily gain did not show a large significance in increasing the odds of pregnancy. However, pubertal status played a large role in increasing the odds of pregnancy. For WCREC heifers, increasing BW gain from breeding to ultrasound increased the odds of pregnancy for heifers not pubertal prior to breeding, making the argument that they are larger, later maturing heifers more valid.

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Table 1. Effect of birth to weaning ADG on pubertal status (GSL)

Mean ADG	SE	Min	Max
1.75	0.35	1.00	2.45

ADG[1]	Odds of Pubertal[2]	*P*-value	Odds Ratio[3]
1.00	3.01	<0.01	0.59
1.25	3.58	<0.01	0.71
1.50	4.26	<0.01	0.84
1.75	5.07	<0.01	NA
2.00	6.03	<0.01	1.19
2.25	7.17	<0.01	1.41
2.45	8.53	<0.01	1.68

[1]Average daily gain (ADG) in pounds per day.
[2]Odds of being pubertal vs. non-pubertal.
[3]Odds ratio, odds of being pubertal at each ADG level compared with the odds of being pubertal at the ADG mark NA.
[4]NA is the mean ADG for current development period.

Table 2. Effect of weaning to breeding ADG on pubertal status (GSL)

Mean ADG	SE	Min	Max
0.91	0.32	0.25	1.50

ADG[1]	Odds of Pubertal[2]	*P*-value	Odds Ratio[3]
0.25	0.94	0.65	0.63
0.50	1.12	0.24	0.75
0.75	1.34	<0.01	0.89
0.91	1.50	<0.01	NA
1.00	1.59	<0.01	1.06
1.25	1.90	<0.01	1.27
1.50	2.27	<0.01	1.51

[1]Average daily gain (ADG) in pounds per day.

[2]Odds of being pubertal vs. non-pubertal.

[3]Odds ratio, odds of being pubertal at each ADG level compared with the odds of being pubertal at the ADG mark NA.

[4]NA is the mean ADG for current development period.

Table 3. Effect of pubertal status on pregnancy rate (GSL)

	Birth to Weaning ADG		
Factor	Odds[2]	Odds Ratio[3]	*P*-value
Pubertal[1]			<0.01
0	4.25	NA	
1	8.26	1.94	
	Weaning to Breeding ADG		
Factor	Odds[2]	Odds Ratio[3]	*P*-value
Pubertal[1]			<0.01
0	4.15	NA	
1	8.32	2.01	
	Breeding to Ultrasound ADG		
Factor	Odds[2]	Odds Ratio[3]	*P*-value
Pubertal[1]			<0.01
0	4.20	NA	
1	7.87	1.87	

[1]Pubertal status: 0-non-pubertal, 1-pubertal.
[2]Odds of being pregnant vs. not pregnant for pubertal status.
[3]Odds ratio, odds of being pregnant if pubertal prior to breeding compared with non-pubertal.

Table 4. Effect of weaning to breeding on pubertal status (WCREC)

Mean ADG	SE	Min	Max
1.61	0.60	0.40	2.80

ADG[1]	Odds[2]	*P*-value	Odds Ratio[3]
0.40	3.33	<0.01	1.46
0.50	3.23	<0.01	1.42
0.75	2.98	<0.01	1.31
1.00	2.76	<0.01	1.22
1.25	2.54	<0.01	1.12
1.50	2.35	<0.01	1.04
1.61	2.27	<0.01	NA
1.75	2.17	<0.01	0.96
2.00	2.00	<0.01	0.88
2.25	1.85	<0.01	0.81
2.50	1.71	<0.01	0.75
2.75	1.58	<0.01	0.70
2.80	1.46	<0.01	0.64

[1]Average daily gain (ADG) in pounds per day.
[2]Odds of being pubertal vs. non-pubertal.
[3]Odds ratio, odds of being pubertal at each ADG level compared with the odds of being pubertal at the ADG mark NA.
[4]NA is the mean ADG for current development period.

Table 5. The effect of one month prior to breeding ADG on pubertal status (WCREC)

Mean ADG	SE	Min	Max
1.69	0.86	0.00	3.40

ADG[1]	Odds[2]	*P*-value	Odd Ratio[3]
0.00	4.09	<0.01	1.78
0.25	3.76	<0.01	1.63
0.50	3.46	<0.01	1.50
0.75	3.16	<0.01	1.37
1.00	2.90	<0.01	1.26
1.25	2.66	<0.01	1.16
1.50	2.44	<0.01	1.06
1.68	2.30	<0.01	NA
1.75	2.24	<0.01	0.97
2.00	2.06	<0.01	0.90
2.25	1.89	<0.01	0.82
2.50	1.74	<0.01	0.76
2.75	1.59	<0.01	0.69
3.0	1.46	<0.01	0.63
3.25	1.34	0.03	0.58
3.40	1.27	0.10	0.55

[1]Average daily gain (ADG) in pounds per day.
[2]Odds of being pubertal vs. non-pubertal.
[3]Odds ratio, odds of being pubertal at each ADG level compared with the odds of being pubertal at the ADG mark NA.
[4]NA is the mean ADG for current development period.

Table 6. The effect of ADG on AI ultrasound to final pregnancy ultrasound on final pregnancy rates (WCREC)

Mean ADG	SE	Min	Max
1.44	0.84	0.60	2.28

ADG[1]	Odds[2]	*P*-value	Odds Ratio[3]
0.60	1.84	<0.01	0.72
0.75	1.95	<0.01	0.76
1.00	2.15	<0.01	0.84
1.25	2.37	<0.01	0.93
1.44	2.55	<0.01	NA[4]
1.75	2.88	<0.01	1.13
2.00	3.18	<0.01	1.25
2.28	3.54	<0.01	1.39

[1]Average daily gain (ADG) in pounds per day.
[2]Odds of being pregnant vs. not pregnant.
[3]Odds ratio, odds of being pregnant at each ADG level compared with the odds of being pregnant at the ADG mark NA.
[4]NA is the mean ADG for current development period.

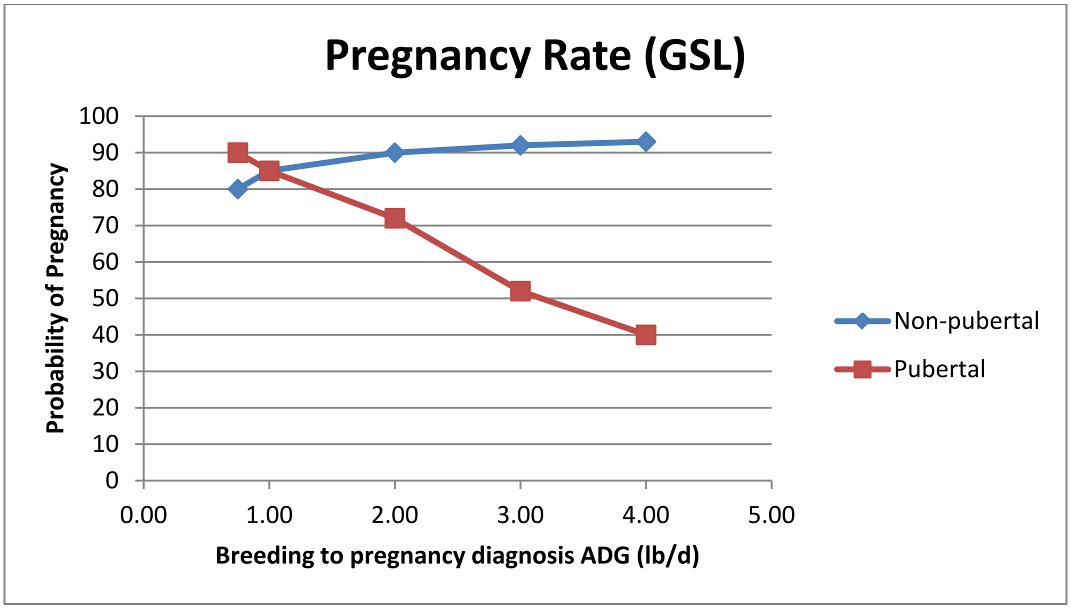

Figure 1. Depicts the interaction (*P* < 0.01) of pubertal status and ADG from breeding to pregnancy diagnosis for heifers at Gudmundsen Sandhills Laboratory (GSL). The Y-axis represents the probability of pregnancy for each ADG (X-axis).

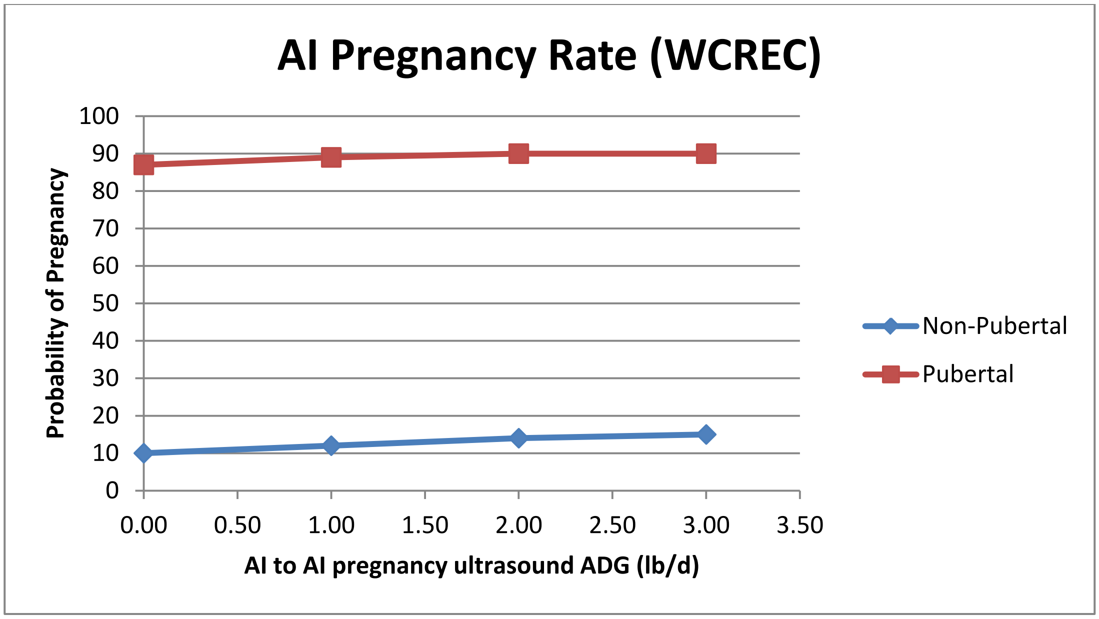

Figure 2. Depicts the effect (*P* = 0.04) of ADG from AI to AI pregnancy ultrasound on AI pregnancy rates for North Platte (WCREC) heifers. The Y axis represents the probability of AI pregnancy for each ADG (X-axis).

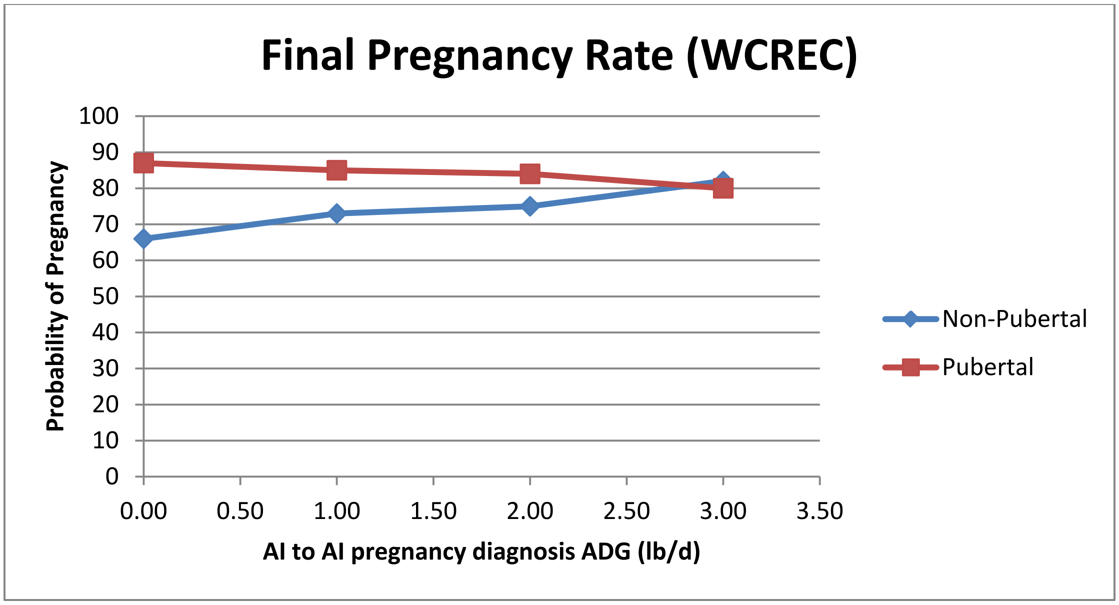

Figure 3. Depicts the interaction (P = 0.06) of ADG from AI to AI pregnancy ultrasound on final pregnancy rates for North Platte (WCREC) heifers. The Y-axis is the probability of final pregnancy at each ADG (X-axis).

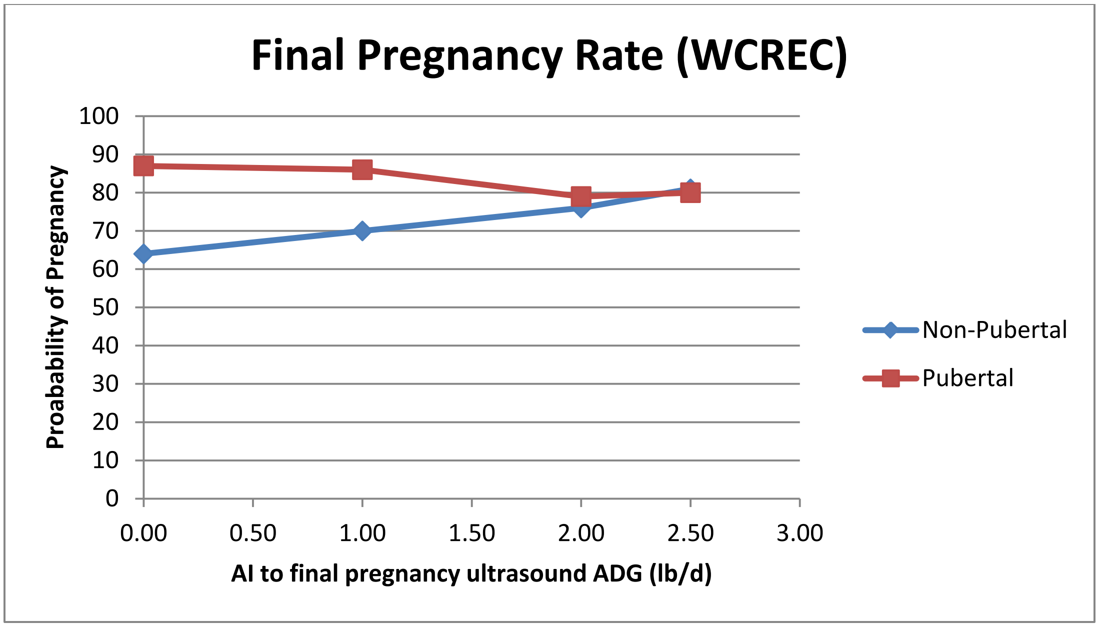

Figure 4. Depicts the interaction (P = 0.06) of ADG from AI to final pregnancy ultrasound on final pregnancy rates for North Platte (WCREC) heifers. The Y-axis is the probability of final pregnancy at each ADG (X-axis).

CHAPTER III:

Comparison of melengestrol acetate and controlled internal drug release long-term progestin-based synchronization protocols on fixed-time AI pregnancy rate in beef heifers

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Abstract

Nulliparous, predominately Angus beef heifers at a commercial ranch in the Nebraska Sandhills were randomly assigned to 1 of 2 progestin-based time AI protocols to compare pregnancy rates. Heifers assigned to melengestrol acetate (**MGA**, n = 688) received MGA (0.5 mg·heifer^{-1}·d^{-1}) from d 0 to 13 and administered $PGF_{2\alpha}$ (25 mg, i.m.) on d 32, fixed-time AI (**FTAI**) occurred approximately 72 h after $PGF_{2\alpha}$. Heifers assigned to 14-d controlled internal drug release (**14-d CIDR**, n = 697) received a CIDR insert (1.38 g of progesterone) from d 2 to 16, followed by administration of $PGF_{2\alpha}$ on d 32, FTAI occurred approximately 66 h after $PGF_{2\alpha}$. All heifers received GnRH (100 µg, i.m.) at FTAI on d 35. Heifers were estrus detected twice daily, d 15 to 25 following FTAI and AI 12 to 18 h after observed estrus. Pregnancy was determined by transrectal ultrasonography 45 d after FTAI, 50 d following second AI, and 36 d following bull removal. Heifers had similar (*P* = 0.49) FTAI pregnancy rates between MGA and 14-d CIDR (62 vs. 61 ± 2%, respectively). A similar (*P* = 0.83) proportion of MGA and 14-d CIDR heifers displayed a second estrus (27 vs. 26 ± 2%, respectively); however, heifers

previously synchronized with MGA tended (*P* = 0.06) to have greater second AI pregnancy rate (66 vs. 56 ± 2%, respectively). Overall pregnancy rate was similar (*P* = 0.27) between MGA and 14-d CIDR treatments (93 vs. 90 ± 1%, respectively). The MGA system was the more cost effective synchronization protocol in this study.

Introduction

Yearling beef heifers are the future of the cowherd and their lifetime reproductive success is dependent on conceiving early in the first and subsequent breeding seasons. Research has indicated beef females that conceive early in the breeding season and calve within the first 21 d of the calving season have increased lifetime reproductive performance and produce progeny with greater overall productivity than those born later in the calving season (Lesmeister et al., 1973; Schafer et al., 1990; Funston et al., 2012). Estrous synchronization and AI are reproductive procedures that can produce a greater proportion of pregnant beef heifers early in the breeding season. Additional benefits from estrous synchronization include, but are not limited to, a shortened calving season resulting in a more uniform calf crop and more rapid genetic improvement (Dziuk and Bellows, 1983). However, there has been limited adoption of estrous synchronization and AI due to time, labor, and cost to implement (NAHMS, 2007-2008).

Implementation of fixed-time AI (**FTAI**) protocols can reduce time and labor inputs. Progestin-based estrous synchronization, such as melengestrol actetate (**MGA**) and controlled internal drug release (**CIDR**) have been documented to induce estrous cyclicity in heifers failing to reach puberty prior to administration (Gonzalez-Padilla et al., 1975; Patterson et al., 1990; Lucy et al., 2001). Kojima et al. (2004) reported decreased synchrony (53 vs. 69%) and AI pregnancy rate (47 vs. 63%) with MGA compared with CIDR. However, Mallory et al. (2010) found MGA and CIDR compare similarly in regards to estrous response, synchronization of estrus, and resulting pregnancy rate.

Combining progestin-based estrous synchronization with CIDR and FTAI (Busch et al., 2007) or MGA vs. 14-d CIDR with estrus detection and AI followed by clean-up FTAI (Tauck et al., 2007) have produced acceptable pregnancy rates. Implementation of strict FTAI can reduce time and labor, by eliminating estrus detection and minimizing the number of times heifers are handled. Therefore, the objectives of this study were to evaluate the pregnancy rates and economic parameters of MGA and 14-d CIDR FTAI protocols in beef heifers.

Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Heifers and Diet

Nulliparous, predominately Angus, yearling beef heifers (n = 1,385) purchased from livestock auctions in Nebraska and South Dakota were utilized in this study, which took place on a commercial ranch in the Nebraska Sandhills. Upon arrival, heifers were vaccinated with Express 3 FP3 VL3 (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) and de-wormed (Safe-Guard, Merck Animal Health, Summit, NJ). Pelvic area was measured and ovaries were palpated for the presence of a significant structure (follicle and/or corpus luteum) by a single technician. Heifers with a small pelvic area, under-developed reproductive tracts, and freemartins were culled (n = 15). Heifer average BW was 329 kg at enrollment. Prior to estrous synchronization treatment, heifers were placed in drylot and offered 7.1 kg/d of a diet containing wet distillers grains plus solubles (26.9% DM), mixed hay (66.9% DM), and a supplement (6.2% DM) during a 14-d adaptation period. After heifers were assigned to treatment groups they were offered 8.6 kg/d of the same diet (Table 1).

Treatments

Heifers from varying sources were placed in 1 large pen and randomly subdivided into 4 groups, then randomly assigned to 1 of 2 treatments: MGA (n = 688) or 14-d CIDR (n = 697; Figure 1). Heifers assigned to MGA received melengestrol acetate (0.5 mg/d per heifer; Pfizer Animal Health) from d 0 through 13 and were administered $PGF_{2\alpha}$ (25 mg i.m.; Lutalyse, Pfizer Animal Health) 19 d after MGA withdrawal (d 32); heifers were AI approximately 72 h after $PGF_{2\alpha}$ (d 35). Heifers assigned to 14-d CIDR received an Eazi-Breed CIDR insert (1.38 g progesterone; Pfizer Animal Health) from d 2 to 16, followed by administration of $PGF_{2\alpha}$ 16d after CIDR removal (d 32), heifers were AI approximately 66 h after $PGF_{2\alpha}$ (d 35). Both treatment groups received GnRH (100 μg i.m.; Factrel, Pfizer Animal Health) at FTAI.

Artificial Insemination, Natural Service, and Pregnancy Diagnosis

Heifers were inseminated by 10 AI technicians using semen from a single bull to reduce sire variation. Following FTAI heifers remained in the drylot and were observed twice daily for signs of estrus d 15 to 25. Heifers observed in estrus were AI 12-18 h later and placed on summer pasture. Heifers not observed in estrus remained in the drylot until pregnancy diagnosis via transrectal ultrasonography 45 d after FTAI. Bulls were placed with heifers approximately 32 d after FTAI for 50 d with a bull to heifer ratio of 1:25. Repeat AI heifers were examined for pregnancy approximately 50 d after second AI. Diagnosis of natural service pregnancy occurred approximately 36 d following removal of bulls.

Economic Analysis

A partial budget analysis was conducted using the procedure by Feuz (1992). The budget analysis was evaluated for the FTAI, second AI, and overall pregnancy. Costs associated with each treatment (MGA, $PGF_{2\alpha}$, GnRH, and CIDR) were derived from the Estrus Synchronization Planner (Beef Reproduction Task Force, 2011), semen and labor costs were based on actual costs. The value of the heifers at the beginning of the study (purchase value) and at pregnancy diagnosis (cull value) was calculated from the Nebraska and South Dakota average price, reported by the USDA – Agricultural Marketing Service (2012) for each corresponding date. Technician labor for the second AI was calculated using a formula by Johnson and Jones (2006); which included the number of heifers being observed, multiplied by number of days observed, raised to the 0.5 power, divided by the number of heifers that received a second AI. Total breeding costs included progestin source, pharmaceuticals, semen, and labor cost per heifer. Total treatment cost per heifer was calculated by adding the purchase price and total breeding cost. The net cost of 1 pregnant heifer was calculated as the difference between total treatment cost per heifer and cull value, divided by pregnancy rate.

Statistical Analysis

The statistical model included estrus synchronization protocol as the fixed effect. Heifer origin and AI technician were included as random variables. Continuous and binomial data were analyzed using the MIXED and GLIMMIX procedure of SAS 9.2 (SAS Institute Inc., Cary, NC), respectively. Means were separated by LSD, and declared different at $P \leq 0.05$.

Results and Discussion

Pregnancy Rates

Fixed-time AI pregnancy rates did not differ ($P = 0.56$) between MGA and 14-d CIDR (62 vs. 61 ± 2%, respectively). These FTAI pregnancy rates were similar to FTAI pregnancy rates reported by Busch et al. (2007) when comparing 14-d CIDR synchronization protocol with 7-d CIDR (CIDR Select vs. CO-Synch+CIDR) ranging from 47 to 62% across 3 locations, with the 14-d CIDR consistently yielding greater pregnancy rates.

Second AI occurred d 15 to 25 following FTAI. Throughout this period a similar number of heifers from each treatment ($P = 0.83$) were observed in estrus and AI; however, heifers previously synchronized with MGA tended ($P = 0.06$) to have greater second AI conception rate (66 vs. 56% ± 2% for MGA and CIDR, respectively).

Natural service pregnancy rate (66 vs. 65 ± 4%) and overall pregnancy rate (93 vs. 90 ± 1%) were similar ($P > 0.27$) between the MGA and 14-d CIDR groups, respectively. Similar pregnancy rates have been reported when comparing14-d progestin-based synchronization protocols (MGA and CIDR), with a period of estrus detection. Tauck et al. (2007) reported similar pregnancy rates when comparing MGA and 14-d CIDR when heifers were detected for estrus 60 h and AI 12 h later followed by a clean-up FTAI at 72 h for heifers not detected in estrus (66% MGA vs. 62% CIDR) and when utilizing estrus detection for 144 h and AI 12 h later (43 to 54% MGA vs. 49 to 53% CIDR; Mallory et al., 2010). From the present study it appears FTAI has the capability to yield similar pregnancy rates when compared with estrus detection and AI utilizing similar synchronization protocols.

Economic Analysis

When comparing MGA and 14-d CIDR with FTAI, the MGA estrus synchronization protocol resulted in approximately a $15 decrease in cost per pregnant heifer (Table 3). This can be attributed mostly to difference in breeding cost and partly to the number of cull heifers. Heifers AI a second time were estrus detected and AI 12-18 h after observation of standing estrus. The MGA system increased cost approximately $2/ pregnant heifer compared with 14-d CIDR when evaluating the economics for second AI. This difference was due to the numerical decrease in pregnancy rate to the second AI in 14-d CIDR heifers and therefore yielded a greater cull value, as a greater proportion of 14-d CIDR heifers were culled compared with MGA heifers. Comparing overall costs for each synchronization method, the MGA system cost approximately $19 less to produce a pregnant heifer compared with 14-d CIDR primarily due to differences in breeding costs between treatments.

Implications

Use of MGA or 14-d CIDR to synchronize estrus in beef heifers resulted in similar pregnancy rates to FTAI and overall pregnancy rates at the end of the breeding season. In the current study it was more economical to produce a pregnant heifer utilizing the MGA system due to decreased synchronization costs. Estrous synchronization protocols combined with FTAI can reduce time and labor inputs, therefore reducing total costs by eliminating estrus detection.

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Appreciation is expressed to ABS Global (DeForest, WI) for technical assistance and Pfizer Animal Health (New York, NY) for donation of CIDR, MGA, Lutalyse, and Factrel. A special thanks to O’Hare Ranches (Johnstown, NE) for the utilization of their facilities, cattle, and their personnel, whom managed the cattle and maintained records throughout the study. Thank you to Dr. Virgil Heyer for his contribution of technical veterinary labor.

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Table 1. Composition and nutrient analysis of drylot diet fed to heifers[1]

Item	% DM
Wet distiller grain	26.9
Mixed hay	66.9
Supplement[2]	6.2
Diet nutrient analysis, %	
CP	15.7
TDN	65.3
Fat	4.8

[1]Nutrient analysis performed by Cattlemen's Nutrition Services, LLC (Lincoln, NE).

[2]Supplement included 10.0% dried distillers grain plus solubles, 48.8% wheat middlings, 39.9% vitamins and minerals, 0.9% urea, 0.4 % trace mineral premix, supplement provided 200 mg·heifer^{-1}·d^{-1} Rumensin (Elanco Animal Health, Greenfield, IN).

Table 2. Reproductive measurements prior to treatment and effect of controlled internal drug release (14-d CIDR) and melengestrol acetate (MGA) synchronization systems on pregnancy rates

Item	Treatment		SEM	*P*-value
	MGA[1]	14-d CIDR[2]		
N	688	697		
Significant structure,[3] %	99	97	1	0.08
Pelvic area, cm^2	159	157	1	0.50
Fixed-time AI pregnancy rate,[4] %	62	61	2	0.56
Heifers receiving 2nd AI, %	26	26	2	0.83
Second AI pregnancy rate,[5] %	66	56	4	0.06
Natural service pregnancy rate,[6] %	66	65	4	0.85
Final pregnancy rate,[6] %	93	90	1	0.27

[1]Received MGA d 0 to 13, followed by $PGF_{2\alpha}$ d 32, GnRH was administered at fixed time-AI, approximately 72 h after $PGF_{2\alpha}$ (d 35).
[2]Received CIDR d 2 to 16, followed by $PGF_{2\alpha}$ d 32, GnRH was administered at fixed time-AI, approximately 66 h after $PGF_{2\alpha}$ (d 35).
[3]Presence of a palpable follicle and/or corpus luteum.
[4]Determined via transrectal ultrasound 45 d following FTAI.
[5]Determined via transrectal ultrasound approximately 50 d following second AI.
[6]Determined via transrectal ultrasound 36 d following bull removal.

Table 3. Cost comparison of controlled internal drug release (14-d CIDR) and melengestrol acetate (MGA) synchronization protocols

	Treatments	
	MGA[1]	14-d CIDR[2]
Fixed Time AI		
N	688	697
MGA, $/heifer	2.80	-
CIDR, $/heifer	-	10.50
PG/GnRH, $/heifer	5.10	5.10
Semen cost, $/straw	15.00	15.00
Technician labor, $/heifer	3.00	3.00
Breeding cost,[3] $/heifer	25.90	33.60
Heifer pregnancy rate, %	62.4	60.8
Total heifer development cost,[4] $/heifer	1,098	1,106
Cull heifer value,[5] $/heifer	430	446
Net cost for 1 pregnant heifer	1,070	1,085
Difference	-15	
Second AI		
N	175	169
Semen cost, $/straw	15.00	15.00
Technician labor, $/heifer	4.96	4.96
Breeding cost,[3] $/heifer	19.96	19.96
Heifer pregnancy rate, %	65.7	55.3
Total heifer development cost,[4] $/heifer	1,092	1,092
Cull heifer value,[5] $/heifer	384	497
Net cost for 1 pregnant heifer	1,078	1,076
Difference		-2
Overall		
N	688	697
MGA, $/heifer	2.80	-
CIDR, $/heifer	-	10.50
PG/GnRH, $/heifer	5.10	5.10
Semen cost, $/straw	15.00	15.00
Technician labor, $/heifer	3.00	3.00
Adjusted second AI cost,[6] $/heifer	5.07	4.83
Breeding cost,[7] $/heifer	30.97	38.43
Heifer pregnancy rate, %	92.6	90.1
Total heifer development cost,[4] $/heifer	1,103	1,111
Cull heifer value,[5] $/heifer	92	110
Net cost for 1 pregnant heifer	1,092	1,111
Difference	-19	

[1]Received MGA d 0 to 13, followed by $PGF_{2\alpha}$ d 32, GnRH was administered at fixed time-AI, approximately 72 h after $PGF_{2\alpha}$ (d 35).
[2]Received CIDR d 2 to 16, followed by $PGF_{2\alpha}$ d 32, GnRH was administered at fixed time-AI, approximately 66 h after $PGF_{2\alpha}$ (d 35).
[3]Includes cost of progestin source, $PGF_{2\alpha}$, GnRH, semen, and technician labor.
[4]Sum of heifer purchase value and total breeding cost.
[5]Cull weight multiplied by the proportion of heifers culled, multiplied by the cull price per hundred weight.
[6]Proportion of heifers receiving second AI multiplied by total breeding cost for second AI.
[7]Sum of the cost of progestin source, pharmaceuticals, semen, technician labor, and adjusted second AI cost.

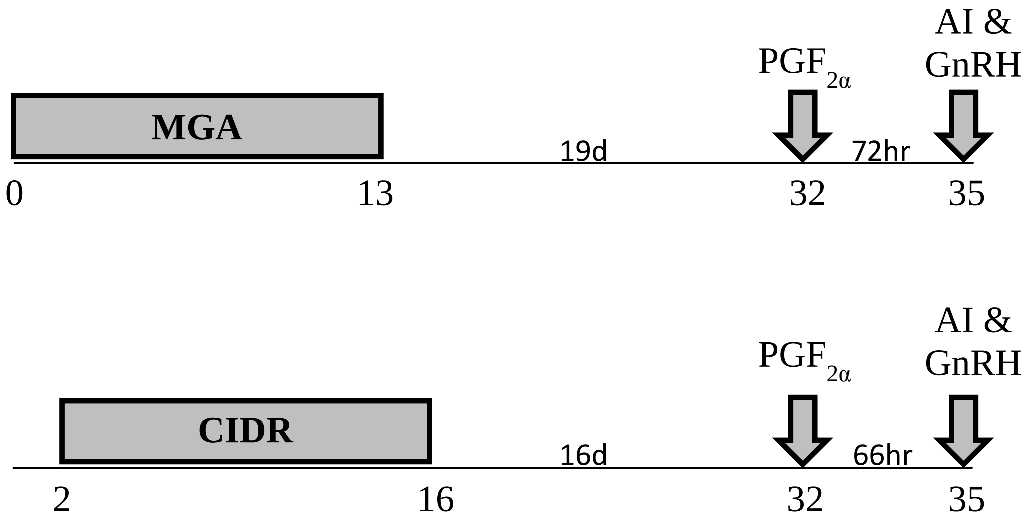

Figure 1. Treatment schedule for heifers assigned to melengestrol acetate (MGA; n = 688; 0.5 mg·heifer^{-1}·d^{-1}; Pfizer Animal Health, New York, NY) from d 0 to 13 or 14-d controlled internal drug release (CIDR; n = 697; 1.38 g progesterone; Pfizer Animal Health) from d 2 to 16. All heifers were administered $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse, Pfizer Animal Health) on d 32. Fixed time AI and GnRH (100 µg i.m.; Factrel, Pfizer Animal Health) injection occurred on d 35 (72 and 66 h following $PGF_{2\alpha}$ administration, for MGA and 14-d CIDR, respectively).